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(54) Title: 26904, 38911, AND 39404, NOVEL SEVEN-TRANSMEMBRANE PROTEINS/G-PROTEIN COUPLED RECEPTORS

(57) Abstract: The present invention relates to newly identified seven-transmembrane proteins, including proteins that function as receptors belonging to the superfamily of G-protein-coupled receptors. The invention also relates to polynucleotides encoding the seven-transmembrane proteins/receptors. The invention further relates to methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides as a target for diagnosis and treatment in seven-transmembrane protein/receptor-mediated and related disorders. The invention further relates to drug-screening methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the seven-transmembrane protein/receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the receptor polypeptides and polynucleotides.

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26904, 38911, AND 39404, NOVEL SEVEN-TRANSMEMBRANE
PROTEINS/G-PROTEIN COUPLED RECEPTORS

FIELD OF THE INVENTION

The present invention relates to newly identified seven-transmembrane proteins, including proteins that function as receptors belonging to the superfamily of G-protein-coupled receptors. The invention also relates to polynucleotides encoding the seven-transmembrane proteins/receptors. The invention further relates to methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides as a target for diagnosis and treatment in seven-transmembrane protein/receptor-mediated and related disorders. The invention further relates to drug-screening methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the seven-transmembrane protein/receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the seven-transmembrane/receptor polypeptides and polynucleotides.

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BACKGROUND OF THE INVENTION

G-protein coupled receptors

G-protein coupled receptors (GPCRs) constitute a major class of proteins responsible for transducing a signal within a cell. GPCRs have three structural domains: an amino terminal extracellular domain, a transmembrane domain containing seven transmembrane segments, three extracellular loops, and three intracellular loops, and a carboxy terminal intracellular domain. Upon binding of a ligand to an extracellular portion of a GPCR, a signal is transduced within the cell that results in a change in a biological or physiological property of the cell. GPCRs, along with G-proteins and effectors (intracellular enzymes and channels

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modulated by G-proteins), are the components of a modular signaling system that connects the state of intracellular second messengers to extracellular inputs.

GPCR genes and gene-products are potential causative agents of disease (Spiegel *et al.*, *J. Clin. Invest.* 92:1119-1125 (1993); McKusick *et al.*, *J. Med. Genet.* 30:1-26 (1993)). Specific defects in the rhodopsin gene and the V2 vasopressin receptor gene have been shown to cause various forms of retinitis pigmentosa (Nathans *et al.*, *Annu. Rev. Genet.* 26:403-424 (1992)), and nephrogenic diabetes insipidus (Holtzman *et al.*, *Hum. Mol. Genet.* 2:1201-1204 (1993)). These receptors are of critical importance to both the central nervous system and peripheral physiological processes. Evolutionary analyses suggest that the ancestor of these proteins originally developed in concert with complex body plans and nervous systems.

The GPCR protein superfamily can be divided into five families: Family I, receptors typified by rhodopsin and the β 2-adrenergic receptor and currently represented by over 200 unique members (Dohlman *et al.*, *Annu. Rev. Biochem.* 60:653-688 (1991)); Family II, the parathyroid hormone/calcitonin/secretin receptor family (Juppner *et al.*, *Science* 254:1024-1026 (1991); Lin *et al.*, *Science* 254:1022-1024 (1991)); Family III, the metabotropic glutamate receptor family (Nakanishi, *Science* 258 597:603 (1992)); Family IV, the cAMP receptor family, important in the chemotaxis and development of *D. discoideum* (Klein *et al.*, *Science* 241:1467-1472 (1988)); and Family V, the fungal mating pheromone receptors such as STE2 (Kurjan, *Annu. Rev. Biochem.* 61:1097-1129 (1992)).

There are also a small number of other proteins which present seven putative hydrophobic segments and appear to be unrelated to GPCRs; they have not been shown to couple to G-proteins. *Drosophila* expresses a photoreceptor-specific protein, bride of sevenless (boss), a seven-transmembrane-segment protein which has been extensively studied and does not show evidence of being a GPCR (Hart *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5047-5051 (1993)). The gene frizzled (*fz*) in *Drosophila* is also thought to be a protein with seven transmembrane segments. Like boss, *fz* has not been shown to couple to G-proteins (Vinson *et al.*, *Nature* 338:263-264 (1989)).

G proteins represent a family of heterotrimeric proteins composed of α , β and γ subunits, that bind guanine nucleotides. These proteins are usually linked to cell surface receptors, e.g., receptors containing seven transmembrane segments. Following ligand binding to the GPCR, a conformational change is transmitted to the G protein, which causes the α -subunit to exchange a bound GDP molecule for a GTP molecule and to dissociate from the $\beta\gamma$ -subunits. The GTP-bound form of the α -subunit typically functions as an effector-modulating moiety, leading to the production of second messengers, such as cAMP (e.g., by activation of adenylyl cyclase), diacylglycerol or inositol phosphates. Greater than 20 different types of α -subunits are known in humans. These subunits associate with a smaller pool of β and γ subunits. Examples of mammalian G proteins include Gi, Go, Gq, Gs and Gt. G proteins are described extensively in Lodish *et al.*, *Molecular Cell Biology*, (Scientific American Books Inc., New York, N.Y., 1995), the contents of which are incorporated herein by reference. GPCRs, G proteins and G protein-linked effector and second messenger systems have been reviewed in *The G-Protein Linked Receptor Fact Book*, Watson *et al.*, eds., Academic Press (1994).

Purinoceptors

Purines, and especially adenosine and adenine nucleotides, have a broad range of pharmacological effects mediated through cell-surface receptors. For a general review, see "Adenosine and Adenine Nucleotides" in *The G-Protein Linked Receptor Facts Book*, Watson *et al.* (Eds.) Academic Press (1994), pp. 19-31.

Some effects of ATP include the regulation of smooth muscle activity, stimulation of the relaxation of intestinal smooth muscle and bladder contraction, stimulation of platelet activation by ADP when released from vascular endothelium, and excitatory effects in the central nervous system. Some effects of adenosine include vasodilation, bronchoconstriction, immunosuppression, inhibition of platelet aggregation, cardiac depression, stimulation of *nociceptive afferants*, inhibition of neurotransmitter release, pre- and postsynaptic depressant action, reducing motor activity, depressing respiration, inducing sleep, relieving anxiety, and inhibition of release of factors, such as hormones.

Distinct receptors exist for adenosine and adenine nucleotides. Clinical actions of such analogs as methylxanthines, for example, theophylline and caffeine, are thought to achieve their effects by antagonizing adenosine receptors. Adenosine has a low affinity for adenine nucleotide receptors, while
5 adenine nucleotides have a low affinity for adenosine receptors.

There are four accepted subtypes of adenosine receptors, designated A₁, A_{2A}, A_{2B}, and A₃. In addition, an A₄ receptor has been proposed based on labeling by 2-phenylaminoadenosine (Cornfield *et al.*, *Mol. Pharmacol.* 42:552-561
(1992)).

10 P_{2X} receptors are ATP-gated cation channels (See *Neuropharmacology* 36 (1977)). The proposed topology for P_{2X} receptors is two transmembrane regions, a large extracellular loop, and intracellular N and C-termini.

Numerous cloned receptors designated P_{2Y} have been proposed to be members of the G-protein coupled family. UDP, UTP, ADP, and ATP have been
15 identified as agonists. To date, P_{2Y1-7} have been characterized although it has been proposed that P_{2Y7} may be a leukotriene B₄ receptor (Yokomizo *et al.*, *Nature* 387:620-624 (1997)). It is widely accepted, however, that P_{2Y 1, 2, 4, and 6} are members of the G-protein coupled family of P_{2Y} receptors.

At least three P₂ purinoceptors from the hematopoietic cell line HEL have
20 been identified by intracellular calcium mobilization and by photoaffinity labeling (Akbar *et al.*, *J. Biochem.* 271:18363-18567 (1996)).

The A₁ adenosine receptor was designated in view of its ability to inhibit adenylyl cyclase. The receptors are distributed in many peripheral tissues such as heart, adipose, kidney, stomach and pancreas. They are also found in peripheral
25 nerves, for example intestine and vas deferens. They are present in high levels in the central nervous system, including cerebral cortex, hippocampus, cerebellum, thalamus, and striatum, as well as in several cell lines. Agonists and antagonists can be found on page 22 of *The G-Protein Linked Receptor Facts Book* cited above, herein incorporated by reference. These receptors are reported to inhibit
30 adenylyl cyclase and voltage-dependent calcium channels and to activate potassium channels through a pertussis-toxin-sensitive G-protein suggested to be of the G_i/G_o

class. A₁ receptors have also been reported to induce activation of phospholipase C and to potentiate the ability of other receptors to activate this pathway.

The A_{2A} adenosine receptor has been found in brain, such as striatum, olfactory tubercle and nucleus accumbens. In the periphery, A₂ receptors mediate vasodilation, immunosuppression, inhibition of platelet aggregation, and gluconeogenesis. Agonists and antagonists are found in *The G-Protein Linked Receptor Facts Book* cited above on page 25, herein incorporated by reference. This receptor mediates activation of adenylylase through G_s.

The A_{2B} receptor has been shown to be present in human brain and in rat intestine and urinary bladder. Agonists and antagonists are discussed on page 27 of *The G-Protein Linked Receptor Facts Book* cited above, herein incorporated by reference. This receptor mediates the stimulation of cAMP through G_s.

The A₃ adenosine receptor is expressed in testes, lung, kidney, heart, central nervous system, including cerebral cortex, striatum, and olfactory bulb. A discussion of agonists and antagonists can be found on page 28 of *The G-Protein Linked Receptor Facts Book* cited above, herein incorporated by reference. The receptor mediates the inhibition of adenylylase through a pertussis-toxin-sensitive G-protein, suggested to be of the G_i/G_o class.

The P_{2Y} purinoceptor shows a similar affinity for ATP and ADP with a lower affinity for AMP. The receptor has been found in smooth muscle, for example, taeni caeci and in vascular tissue where it induces vasodilation through endothelium-dependent release of nitric oxide. It has also been shown in avian erythrocytes. Agonists and antagonists are discussed on page 30 of *The G-Protein Linked Receptor Facts Book* cited above, herein incorporated by reference. The receptor function through activation of phosphoinositide metabolism through a pertussis-toxin-insensitive G-protein, suggested to be of the G_i/G_o class.

Receptor for Human C5a Anaphylatoxin

Chemotaxis of phagocytic cells is a key event in host defense and inflammatory responses. The C5a receptor mediates the pro-inflammatory and chemotaxis actions of the complement anaphylatoxin C5a. This receptor stimulates chemotaxis granule enzyme release, superoxide anion production, and

upregulates expression and activity of the adhesion molecule MAC-1 and of CR-1, and mediates a decrease in cell surface glycoprotein 100, MEL-14, in anaphylaxis and in septic shock. This receptor is a member of the rhodopsin superfamily of receptors. In contrast to other receptors of this family (adrenergic, serotonergic, dopaminergic, FSH/LH, substance P and substance K), the C5a receptor functions in a concentration gradient of ligand and internalizes bound receptor during chemotaxis.

Accordingly, GPCRs, and especially complement receptors and purinoceptors, are a major target for drug action and development. Accordingly, it is valuable to the field of pharmaceutical development to identify and characterize previously unknown GPCRs. The present invention advances the state of the art by providing novel seven-transmembrane proteins/GPCRs, including a previously unidentified human seven-transmembrane protein/GPCR having homology to purinoceptors.

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SUMMARY OF THE INVENTION

It is an object of the invention to identify novel seven-transmembrane proteins/GPCRs.

It is a further object of the invention to provide novel seven-transmembrane protein/GPCR polypeptides that are useful as reagents or targets in seven-transmembrane protein/receptor assays applicable to treatment and diagnosis of seven-transmembrane protein/GPCR-mediated disorders.

It is a further object of the invention to provide polynucleotides corresponding to the novel seven-transmembrane protein/GPCR receptor polypeptides that are useful as targets and reagents in seven-transmembrane protein/receptor assays applicable to treatment and diagnosis of seven-transmembrane protein/GPCR-mediated disorders and useful for producing novel seven-transmembrane protein/receptor polypeptides by recombinant methods.

A specific object of the invention is to identify compounds that act as agonists and antagonists and modulate the expression of the novel seven-transmembrane proteins/receptors.

A further specific object of the invention is to provide compounds that modulate expression of the seven-transmembrane proteins/receptors for treatment and diagnosis of seven-transmembrane protein/GPCR- related disorders.

The invention is thus based on the identification of novel seven-
5 transmembrane proteins/GPCRs, designated 39404, 38911, and 26904. As discussed more fully below, 39404 contains sequence homology or motifs/signatures that classify this protein in the GPCR superfamily, as a member of the rhodopsin and metabotropic families of G-protein coupled receptors.

The invention provides isolated 39404 polypeptides including a
10 polypeptide having the amino acid sequence shown in SEQ ID NO:1, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1847 on May 9, 2000.

The invention provides isolated 38911 polypeptides including a
15 polypeptide having the amino acid sequence shown in SEQ ID NO:3, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1654 on April 6, 2000.

The invention provides isolated 26904 polypeptides including a
polypeptide having the amino acid sequence shown in SEQ ID NO:5.

The invention also provides isolated 39404 nucleic acid molecules having
20 the sequence shown in SEQ ID NO:2 or in the corresponding deposited cDNA.

The invention also provides isolated 38911 nucleic acid molecules having
the sequence shown in SEQ ID NO:4 or in the corresponding deposited cDNA.

The invention also provides isolated 26904 nucleic acid molecules having
the sequence shown in SEQ ID NO:6.

25 The invention also provides variant polypeptides having an amino acid sequence that is substantially homologous to the amino acid sequence shown in SEQ ID NO:1 or encoded by the deposited cDNA.

The invention also provides variant polypeptides having an amino acid
sequence that is substantially homologous to the amino acid sequence shown in
30 SEQ ID NO:3 or encoded by the deposited cDNA.

The invention also provides variant polypeptides having an amino acid sequence that is substantially homologous to the amino acid sequence shown in SEQ ID NO:5 or encoded by the deposited cDNA.

5 The invention also provides variant nucleic acid sequences that are substantially homologous to the nucleotide sequence shown in SEQ ID NO:2 or in the deposited cDNA.

The invention also provides variant nucleic acid sequences that are substantially homologous to the nucleotide sequence shown in SEQ ID NO:4 or in the deposited cDNA.

10 The invention also provides variant nucleic acid sequences that are substantially homologous to the nucleotide sequence shown in SEQ ID NO:6 or in the deposited cDNA.

The invention also provides fragments of the polypeptide shown in SEQ ID NO:1 and nucleotide sequence shown in SEQ ID NO:2, as well as substantially
15 homologous fragments of the polypeptide or nucleic acid.

The invention also provides fragments of the polypeptide shown in SEQ ID NO:3 and nucleotide sequence shown in SEQ ID NO:4, as well as substantially homologous fragments of the polypeptide or nucleic acid.

20 The invention also provides fragments of the polypeptide shown in SEQ ID NO:5 and nucleotide sequence shown in SEQ ID NO:6, as well as substantially homologous fragments of the polypeptide or nucleic acid.

The invention further provides nucleic acid constructs comprising the nucleic acid molecules described above. In a preferred embodiment, the nucleic acid molecules of the invention are operatively linked to a regulatory sequence.

25 The invention also provides vectors and host cells for expressing the nucleic acid molecules and polypeptides of the invention and particularly recombinant vectors and host cells.

The invention also provides methods of making the vectors and host cells and methods for using them to produce the nucleic acid molecules and
30 polypeptides of the invention.

The invention also provides antibodies or antigen-binding fragments thereof that selectively bind the polypeptides and fragments of the invention.

The invention also provides methods of screening for compounds that modulate expression or activity of the polypeptides or nucleic acid (RNA or DNA) of the invention.

5 The invention also provides a process for modulating polypeptide or nucleic acid expression or activity, especially using the screened compounds. Modulation may be used to treat conditions related to aberrant activity or expression of the polypeptides or nucleic acids of the invention.

The invention also provides assays for determining the presence or absence of and level of the polypeptides or nucleic acid molecules of the invention
10 in a biological sample, including for disease diagnosis.

The invention also provides assays for determining the presence of a mutation in the polypeptides or nucleic acid molecules, including for disease diagnosis.

In still a further embodiment, the invention provides a computer readable
15 means containing the nucleotide and/or amino acid sequences of the nucleic acids and polypeptides of the invention.

DESCRIPTION OF THE DRAWINGS

20 Figure 1 shows the 39404 nucleotide sequence (SEQ ID NO:2) and the deduced 39404 amino acid sequence (SEQ ID NO:1).

Figure 2 shows a 39404 protein hydrophobicity plot. The amino acids correspond to 1-337 and show the seven transmembrane segments.

Figure 3 shows an analysis of the 39404 amino acid sequence: α turn and
25 coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability plot.

Figure 4 shows an analysis of the 39404 open reading frame for amino acids corresponding to specific functional sites. Glycosylation sites are shown in the figure with the actual modified residue being the first amino acid. cAMP- and
30 cGMP-dependent protein kinase phosphorylation sites are shown in the figure with the actual modified residue being the last amino acid. Protein kinase C phosphorylation sites shown in the figure with the actual modified residue being

the first amino acid. A casein kinase II phosphorylation site is shown in the figure with the actual modified residue being the first amino acid. In addition, amino acids corresponding in position to the GPCR signature and containing the invariant arginine are found in the sequence FRY at amino acids 130-132. This figure also

5 shows transmembrane segments predicted by MEMSAT for the predicted entire coding sequence and for a predicted mature peptide. For example, for the entire coding sequence, it is predicted that amino acids 1 to about 37 constitute the amino terminal extracellular domain, amino acids about 38-305 constitute the region spanning the transmembrane domain, and amino acids about 306-337 constitute

10 the carboxy terminal intracellular domain. The transmembrane domain contains seven transmembrane segments, three extracellular loops and three intracellular loops. The transmembrane segments are found from about amino acid 38 to about amino acid 60, from about amino acid 70 to about amino acid 90, from about amino acid 117 to about amino acid 136, from about amino acid 149 to about

15 amino acid 172, from about amino acid 200 to about amino acid 222, from about amino acid 242 to about amino acid 260, and from about amino acid 283 to about amino acid 305. Within the region spanning the entire transmembrane domain are three intracellular and three extracellular loops. The three intracellular loops are found from about amino acid 61 to about amino acid 69, from about amino acid

20 137 to about amino acid 148, and from about amino acid 223 to about amino acid 241. The three extracellular loops are found at from about amino acid 91 to about amino acid 116, from about amino acid 173 to about amino acid 199, and from about amino acid 261 to about amino acid 282.

Figure 5 shows expression of the 39404 protein in normal human tissues.

25 Figure 6 shows expression of the 39404 protein in human cardiovascular tissues. Int prolif: intimal proliferation; Int mamm: internal mammary; CHF: congestive heart failure; ISCH: ischemia; Myop: myopathy.

Figure 7 shows expression of the protein in human cardiovascular tissues.

Figure 8 shows the 38911 nucleotide sequence (SEQ ID NO:4) and the

30 deduced 38911 amino acid sequence (SEQ ID NO:3).

Figure 9 shows an analysis of the 38911 amino acid sequence: α turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability plot.

Figure 10 shows a 38911 protein hydrophobicity plot. The amino acids
5 correspond to 1-337 and show the seven transmembrane segments.

Figure 11 shows an analysis of the 38911 open reading frame for amino acids corresponding to specific functional sites. A glycosylation site is found at amino acids 3-6. A cAMP- and cGMP-dependent protein kinase phosphorylation site is found at amino acids 324-327. A protein kinase C phosphorylation site is
10 found at amino acids 17-19. A second protein kinase C phosphorylation site is found at amino acids 323-325. Casein kinase II phosphorylation sites are found at amino acids 194-197, 327-330, and 333-336. N-myristoylation sites are found at amino acids 26-31, 49-54, 103-108, 150-155, 156-161, 191-196, 253-258, 278-283, and 316-321. For the cAMP and cGMP dependent protein kinase
15 phosphorylation, the actual modified residue is the last amino acid. For protein kinase C phosphorylation, the actual modified residue is the first amino acid. For casein kinase II phosphorylation, the actual modified residue is the first amino acid. For N-myristoylation, the actual modified residue is the first amino acid.

It is predicted that amino acids 1 to about 40 constitute the amino terminal
20 extracellular domain, amino acids about 41-294 constitute the region spanning the transmembrane domain, and amino acids about 259-337 constitute the carboxy terminal intracellular domain. The transmembrane domain contains seven transmembrane segments, three extracellular loops and three intracellular loops.

The transmembrane segments are found from about amino acid 41 to about amino
25 acid 60, from about amino acid 68 to about amino acid 92, from about amino acid 113 to about amino acid 137, from about amino acid 153 to about amino acid 172, from about amino acid 205 to about amino acid 228, from about amino acid 237 to about amino acid 260, and from about amino acid 275 to about amino acid 294.

Within the region spanning the entire transmembrane domain are three
30 intracellular and three extracellular loops. The three intracellular loops are found from about amino acid 61 to about amino acid 67, from about amino acid 138 to about amino acid 152, and from about amino acid 229 to about amino acid 236.

The three extracellular loops are found at from about amino acid 93 to about amino acid 112, from about amino acid 173 to about amino acid 204, and from about amino acid 261 to about amino acid 274.

Figure 12 shows expression of the 38911 protein in various normal human tissues, using fetal heart as a reference.

Figure 13 shows expression of the 38911 protein in various normal human tissues and in biopsies from fibrotic livers.

Figure 14 shows the 26904 nucleotide sequence (SEQ ID NO:6) and the deduced 26904 amino acid sequence (SEQ ID NO:5).

Figure 15 shows an analysis of the 26904 amino acid sequence: α turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability plot.

Figure 16 shows a 26904 protein hydrophobicity plot. The amino acids show the seven transmembrane segments.

Figure 17 shows an analysis of the 26904 open reading frame for amino acids corresponding to specific functional sites. A glycosylation site is found at amino acids 312-315. A cAMP- and cGMP-dependent protein kinase phosphorylation site is found at amino acids 143-146. Protein kinase C phosphorylation sites are found at about amino acids 6-8, 136-138, 234-236, 245-247, 314-316, 436-438, and 446-448. Casein kinase II phosphorylation sites are found at about amino acids 55-58, 167-170, 218-221, 239-242, 284-287, 416-419, and 447-450. Tyrosine kinase phosphorylation sites are found at about amino acids 118-125, 336-343, 382-389, and 409-415. N-myristoylation sites are found at about amino acids 36-41, 91-96, 261-266, 304-309, 365-370, 404-409, and 420-425. An amidation site is found at about amino acids 141-144. An ATP/GTP-binding site motif A (P-loop) is found at about amino acids 230-237. In the case of protein kinase C phosphorylation, the actual modified residue is the first amino acid. In the case of casein kinase II phosphorylation, the actual modified residue is the first amino acid. In the case of the tyrosine kinase phosphorylation, the modified amino acid is the last amino acid. In the case of N-myristoylation, the modified amino acid is the first amino acid.

It is predicted that amino acids 1 to about 30 constitute the amino terminal extracellular domain, amino acids about 30-435 constitute the region spanning the transmembrane domain, and amino acids about 435-450 constitute the carboxy terminal intracellular domain. The transmembrane domain contains seven
 5 transmembrane segments, three extracellular loops and three intracellular loops. The transmembrane segments are found from about amino acid 30 to about amino acid 50, from about amino acid 100 to about amino acid 120, from about amino acid 140 to about amino acid 165, from about amino acid 200 to about amino acid 240, from about amino acid 305 to about amino acid 340, from about amino acid
 10 360 to about amino acid 380, and from about amino acid 410 to about amino acid 450. Within this region spanning the entire transmembrane domain are three intracellular and three extracellular loops.

Figure 18 shows the expression of 38911 in the following tissues: normal human lung (column 1), normal human kidney (column 2), normal human brain
 15 (column 3), normal human granulocytes (column 4), normal human heart (column 5), normal human spleen (column 6), normal human fetal liver (column 7), a pool of 7 normal human livers (column 8), resting normal human dermal fibroblasts (column 9), normal human lung fibroblasts (column 10), normal human lung fibroblasts cultured for 48 hours with TGF- β (column 11), human fibrotic liver
 20 (columns 12-15), normal human tonsils (column 16), proinflammatory type 1T helper cells (column 17 and 19) and proinflammatory type 2T cells (column 18 and 20). 38911 was expressed at high levels in kidney, spleen, fetal liver, fibrotic liver, and tonsils, and at moderate levels in lung, brain, granulocytes, heart, and normal liver. Expression levels were determined by quantitative PCR (Taqman®
 25 brand quantitative PCR kit, Applied Biosystems). The quantitative PCR reactions were performed according to the kit manufacturer's instructions.

Figure 19 shows the expression of 38911 in the following tissues: CD4+ cells (column 1), CD8+ cells (column 2), resting CD14+ monocytes (column 3), resting peripheral blood mononuclear cells (column 4), CD19+ cells (column 5),
 30 resting CD3+ cells (column 6), bone marrow mononuclear cells (column 7), mobilized peripheral blood CD34+ cells (column 8), adult bone marrow CD34+ cells (column 9), human cord blood CD34+ cells (column 10), human erythroid

cells (column 11), human megakaryocytes (column 12), cultured day 14 neutrophils (column 13), mobilized bone marrow CD15+ cells (column 14), GPA+ cells from human bone marrow (column 15), HepG2.2 cells transfected with hepatitis B virus (column 16), hepatitis B virus-infected liver (column 17),
 5 hepatoma Hep3B cells cultured with normal oxygen level (column 18), hepatoma Hep3B cells cultured with low levels of oxygen (column 19). 38911 expression levels were determined as described in the figure legend for Figure 18.

DETAILED DESCRIPTION OF THE INVENTION

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Receptor function/signal pathway

The 39404 38911, and 26904 receptor proteins are GPCR-like proteins that participate in signaling pathways. As used herein, a "signaling pathway" refers to the modulation (e.g., stimulation or inhibition) of a cellular
 15 function/activity upon the binding of a ligand to the GPCR (39404, 38911, or 26904 protein). Examples of such functions include mobilization of intracellular molecules that participate in a signal transduction pathway, e.g., phosphatidylinositol 4,5-bisphosphate (PIP₂), inositol 1,4,5-triphosphate (IP₃) and adenylyate cyclase; polarization of the plasma membrane; production or secretion
 20 of molecules; alteration in the structure of a cellular component; cell proliferation, e.g., synthesis of DNA; cell migration; cell differentiation; and cell survival. The 39404 protein is expressed in the tissues shown in Figures 5-7. Therefore, cells participating in a 39404 protein signaling pathway include, but are not limited to, cells derived from these tissues, especially those tissues in which the gene is
 25 highly expressed, such as brain, kidney, aortic intimal proliferations, and internal mammary artery. Since the 38911 protein is expressed in the tissues shown in Figures 12 and 13, cells participating in a 38911 protein signaling pathway include, but are not limited to, cells derived from these tissues, especially those cells or tissues in which the gene is highly expressed, such as osteoclasts, spleen,
 30 liver, kidney, tonsils, and testis. The gene is also expressed in CD4⁺ cells (T-lymphocytes), in peripheral blood monocytes, and in neutrophils. Since the 26904 protein is expressed in brain, cells participating in a 26904 protein signaling pathway include, but are not limited to, cells derived from this tissue.

The response mediated by a receptor protein depends on the type of cell. For example, in some cells, binding of a ligand to the receptor protein may stimulate an activity such as release of compounds, gating of a channel, cellular adhesion, migration, differentiation, etc., through phosphatidylinositol or cyclic AMP metabolism and turnover while in other cells, the binding of the ligand will produce a different result. Regardless of the cellular activity/response modulated by the receptor protein, it is universal that a GPCR of the invention interacts with G proteins to produce one or more secondary signals, in a variety of intracellular signal transduction pathways, e.g., through phosphatidylinositol or cyclic AMP metabolism and turnover, in a cell.

As used herein, "phosphatidylinositol turnover and metabolism" refers to the molecules involved in the turnover and metabolism of phosphatidylinositol 4,5-bisphosphate (PIP₂) as well as to the activities of these molecules. PIP₂ is a phospholipid found in the cytosolic leaflet of the plasma membrane. Binding of ligand to the receptor activates, in some cells, the plasma-membrane enzyme phospholipase C that in turn can hydrolyze PIP₂ to produce 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃). Once formed IP₃ can diffuse to the endoplasmic reticulum surface where it can bind an IP₃ receptor, e.g., a calcium channel protein containing an IP₃ binding site. IP₃ binding can induce opening of the channel, allowing calcium ions to be released into the cytoplasm. IP₃ can also be phosphorylated by a specific kinase to form inositol 1,3,4,5-tetraphosphate (IP₄), a molecule which can cause calcium entry into the cytoplasm from the extracellular medium. IP₃ and IP₄ can subsequently be hydrolyzed very rapidly to the inactive products inositol 1,4-bisphosphate (IP₂) and inositol 1,3,4-triphosphate, respectively. These inactive products can be recycled by the cell to synthesize PIP₂. The other second messenger produced by the hydrolysis of PIP₂, namely 1,2-diacylglycerol (DAG), remains in the cell membrane where it can serve to activate the enzyme protein kinase C. Protein kinase C is usually found soluble in the cytoplasm of the cell, but upon an increase in the intracellular calcium concentration, this enzyme can move to the plasma membrane where it can be activated by DAG. The activation of protein kinase C in different cells results in various cellular responses such as the phosphorylation of glycogen synthase, or the

phosphorylation of various transcription factors, e.g., NF-kB. The language "phosphatidylinositol activity", as used herein, refers to an activity of PIP₂ or one of its metabolites.

Another signaling pathway in which a receptor protein of the invention may participate is the cAMP turnover pathway. As used herein, "cyclic AMP turnover and metabolism" refers to the molecules involved in the turnover and metabolism of cyclic AMP (cAMP) as well as to the activities of these molecules. Cyclic AMP is a second messenger produced in response to ligand-induced stimulation of certain G protein coupled receptors. In the cAMP signaling pathway, binding of a ligand to a GPCR can lead to the activation of the enzyme adenylyl cyclase, which catalyzes the synthesis of cAMP. The newly synthesized cAMP can in turn activate a cAMP-dependent protein kinase. This activated kinase can phosphorylate a voltage-gated potassium channel protein, or an associated protein, and lead to the inability of the potassium channel to open during an action potential. The inability of the potassium channel to open results in a decrease in the outward flow of potassium, which normally repolarizes the membrane of a neuron, leading to prolonged membrane depolarization.

Polypeptides

The invention is based on the identification of novel seven-transmembrane proteins/G-coupled protein receptors. Specifically, an expressed sequence tag (EST) was selected based on homology to G-protein-coupled receptor sequences or motifs (e.g., seven-transmembrane domains). This EST was used to design primers based on sequences that it contains and used to identify a 39404 cDNA from a human colon cDNA library, a 38911 cDNA from a human bone marrow cDNA library, and a 26904 cDNA from a human brain cDNA library. Positive clones were sequenced and the overlapping fragments were assembled. Analysis of the assembled sequences revealed that the cloned cDNA molecules encode G-protein coupled receptors (39404, 38911, 26904).

The invention thus relates to a novel GPCR having the deduced amino acid sequence shown in Figure 1 (SEQ ID NO:1) or having the amino acid sequence

encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1847.

5 The invention also thus relates to a novel putative GPCR having the deduced amino acid sequence shown in Figure 8 (SEQ ID NO:3) or having the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1654.

The invention also thus relates to a novel putative GPCR having the deduced amino acid sequence shown in Figure 14 (SEQ ID NO:5).

10 Plasmids containing the nucleotide sequences of the invention were deposited with the Patent Depository of the American Type Culture Collection (ATCC), Manassas, Virginia. The deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms. The deposits are provided as a convenience to those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

15 The deposited sequences, as well as the polypeptides encoded by the sequences, are incorporated herein by reference and control in the event of any conflict, such as a sequencing error, with description in this application.

The "39404 polypeptide" or "39404 protein" refers to the polypeptide in SEQ ID NO:1 or encoded by the deposited cDNA. The "38911 polypeptide" or

20 "38911 protein" refers to the polypeptide in SEQ ID NO:3 or encoded by the deposited cDNA. The "26904 polypeptide" or "26904 protein" refers to the polypeptide in SEQ ID NO:5 or encoded by the deposited cDNA. The term "protein" or "polypeptide", however, further includes the numerous variants of 39404, 38911, or 26904 polypeptides described herein, as well as fragments

25 derived from the full length 39404, 38911, or 26904 polypeptides and variants.

The present invention thus provides isolated or purified 39404, 38911, and 26904 polypeptides and variants and fragments thereof.

The 39404 polypeptide is a 319 residue protein exhibiting three main structural domains, an amino terminal extracellular domain, transmembrane

30 domain, and carboxy terminal intracellular domain, as shown in Figure 4. Based on a BLAST search, highest homology was shown to purinoceptors (rhodopsin superfamily)

The 38911 polypeptide is a 337 residue protein exhibiting three main structural domains, the amino terminal extracellular domain, transmembrane domain, and carboxy terminal intracellular domain, as shown in Figure 11.

Based on a BLAST search, highest homology was shown to the C5a

5 anaphylatoxin receptor (G-protein Linked Receptor Facts Book, Watson and Arkinstall, Editors, Academic Press (1994) New York, pgs. 71-73, incorporated herein by reference for its teachings regarding this receptor).

The 26904 polypeptide is a 450 residue protein exhibiting three main structural domains, the amino terminal extracellular domain, transmembrane
10 domain, and carboxy terminal intracellular domain, as shown in Figure 11.

As used herein, a polypeptide is said to be "isolated" or "purified" when it is substantially free of cellular material when it is isolated from recombinant and non-recombinant cells, or free of chemical precursors or other chemicals when it is chemically synthesized. A polypeptide, however, can be joined to another
15 polypeptide with which it is not normally associated in a cell and still be considered "isolated" or "purified."

The polypeptides of the invention can be purified to homogeneity. It is understood, however, that preparations in which the polypeptide is not purified to homogeneity are useful and considered to contain an isolated form of the
20 polypeptide. The critical feature is that the preparation allows for the desired function of the polypeptide, even in the presence of considerable amounts of other components. Thus, the invention encompasses various degrees of purity.

In one embodiment, the language "substantially free of cellular material" includes preparations of the polypeptide having less than about 30% (by dry
25 weight) other proteins (i.e., contaminating protein), less than about 20% other proteins, less than about 10% other proteins, or less than about 5% other proteins. When the polypeptide is recombinantly produced, it can also be substantially free of culture medium, i.e., culture medium represents less than about 20%, less than about 10%, or less than about 5% of the volume of the protein preparation.

30 A polypeptide is also considered to be isolated when it is part of a membrane preparation or is purified and then reconstituted with membrane vesicles or liposomes.

The language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide in which it is separated from chemical precursors or other chemicals that are involved in its synthesis. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide having less than about 30% (by dry weight) chemical precursors or other chemicals, less than about 20% chemical precursors or other chemicals, less than about 10% chemical precursors or other chemicals, or less than about 5% chemical precursors or other chemicals.

In one embodiment, the 39404 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1. However, the invention also encompasses sequence variants. Variants include a substantially homologous protein encoded by the same genetic locus in an organism, i.e., an allelic variant. Variants also encompass proteins derived from other genetic loci in an organism, but having substantial homology to the 39404 protein of SEQ ID NO:1. Variants also include proteins substantially homologous to the 39404 protein but derived from another organism, i.e., an ortholog. Variants also include proteins that are substantially homologous to the 39404 protein that are produced by chemical synthesis. Variants also include proteins that are substantially homologous to the 39404 protein that are produced by recombinant methods. It is understood, however, that variants exclude any amino acid sequences disclosed prior to the invention.

In another embodiment, the 38911 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3. However, the invention also encompasses sequence variants. Variants include allelic variants. Variants also encompass proteins derived from other genetic loci in an organism, but having substantial homology to the 38911 protein of SEQ ID NO:3. Variants also include substantially homologous orthologs. Variants also include proteins that are substantially homologous to the 38911 protein that are produced by chemical synthesis. Variants also include proteins that are substantially homologous to the 38911 protein that are produced by recombinant methods. It is understood, however, that variants exclude any amino acid sequences disclosed prior to the invention.

In another embodiment, the 26904 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5. However, the invention also encompasses sequence variants. Variants include allelic variants. Variants also encompass proteins derived from other genetic loci in an organism, but having substantial
5 homology to the 26904 protein of SEQ ID NO:5. Variants also include substantially homologous orthologs. Variants also include proteins that are substantially homologous to the 26904 protein that are produced by chemical synthesis. Variants also include proteins that are substantially homologous to the 26904 protein that are produced by recombinant methods. It is understood,
10 however, that variants exclude any amino acid sequences disclosed prior to the invention.

As used herein, two proteins (or a region of the proteins) are substantially homologous to the 39404 protein when the amino acid sequences are at least about 40-45%, 45-50%, 50-55%, 55-60%, typically at least about 60-65%, 65-70%, or
15 70-75%, more typically at least about 70-75%, 75-80%, or 80-85%, and most typically at least about 85-90% or 90-95% or more homologous. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence hybridizing to the nucleic acid sequence, or portion thereof, of the sequence shown in SEQ ID NO:2 under stringent conditions
20 as more fully described below.

As used herein, two proteins (or a region of the proteins) are substantially homologous to the 38911 protein when the amino acid sequences are at least about 35-40%, 40-45%, 45-50%, 55-60%, 60-65%, 65-70%, typically at least about 70-75%, more typically at least about 75-80% or 80-85%, and most typically at least
25 about 85-90% or 90-95% or more homologous. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence hybridizing to the nucleic acid sequence, or portion thereof, of the sequence shown in SEQ ID NO:4 under stringent conditions as more fully described below.

30 As used herein, two proteins (or a region of the proteins) are substantially homologous to the 26904 protein when the amino acid sequences are at least about 50-55%, 55-60%, 60-65%, 65-70%, typically at least about 70-75%, more

typically at least about 75-80% or 80-85%, and most typically at least about 85-90% or 90-95% or more homologous. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence hybridizing to the nucleic acid sequence, or portion thereof, of the sequence shown in SEQ ID NO:6 under stringent conditions as more fully described below.

The invention also encompasses polypeptides having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions (e.g. G protein coupled signaling) performed by the 39404, 38911, or 26904 polypeptides. Similarity is determined by conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Conservative substitutions are likely to be phenotypically silent. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie *et al.*, *Science* 247:1306-1310 (1990).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine
	Tryptophan
	Tyrosine
Hydrophobic	Leucine
	Isoleucine
	Valine
Polar	Glutamine
	Asparagine
Basic	Arginine
	Lysine
	Histidine
Acidic	Aspartic Acid
	Glutamic Acid
Small	Alanine
	Serine
	Threonine
	Methionine
	Glycine

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of

the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the
5 molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two
10 sequences.

The comparison of sequences and determination of percent identity and similarity between two sequences can be accomplished by well-known methods such as using a mathematical algorithm. (*Computational Molecular Biology*, Lesk, A.M., Ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., Ed., Academic Press, New York, 1993;
15 *Computer Analysis of Sequence Data*, Part 1, Griffin, A.M., and Griffin, H.G., Eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., Eds., M Stockton Press, New York, 1991).

20 A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of
25 the respective programs (e.g., NBLAST) can be used. See www.ncbi.nlm.nih.gov. In one embodiment, parameters for sequence comparison can be set at score= 100, wordlength = 12, or can be varied (e.g., W=5 or W=20).

In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman *et al.* (1970) (*J. Mol. Biol.* 48:444-
30 453) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length

weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux *et al.* (1984) *Nucleic Acids Res.* 12(1):387) (available at www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the CGC sequence alignment software package.

When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis *et al.* (1994) *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson *et al.* (1988) *PNAS* 85:2444-8.

A variant polypeptide can differ in amino acid sequence by one or more substitutions, deletions, insertions, inversions, fusions, and truncations or a combination of any of these.

Variant polypeptides can be fully functional or can lack function in one or more activities. Thus, in the present case, variations can affect the function, for example, of one or more of the regions corresponding to ligand binding, membrane association, G-protein binding and signal transduction.

Fully functional variants typically contain only conservative variation or variation in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids which result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree.

Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

As indicated, variants can be naturally-occurring or can be made by recombinant means or chemical synthesis to provide useful and novel

characteristics for the polypeptide. This includes preventing immunogenicity from pharmaceutical formulations by preventing protein aggregation.

Useful variations further can include alteration of ligand binding characteristics. For example, one embodiment involves a variation at the binding
5 site that results in binding but not release, or slower release, of ligand. A further useful variation at the same sites can result in a higher affinity for ligand. Useful variations also include changes that provide for affinity for another ligand. Another useful variation can include one that allows binding but which prevents activation by the ligand. Another useful variation includes variation in the
10 transmembrane G-protein-binding/signal transduction domain that provides for reduced or increased binding by the appropriate G-protein or for binding by a different G-protein than the one with which the receptor is normally associated. Another useful variation provides a fusion protein in which one or more domains or subregions is operationally fused to one or more domains or subregions from
15 another G-protein coupled receptor.

Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham *et al.*, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule.
20 The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992); de Vos *et al.* *Science* 255:306-312 (1992)).

25 Substantial homology can be to the entire nucleic acid or amino acid sequence or to fragments of these sequences.

The invention thus also includes polypeptide fragments of the 39404 protein. Fragments can be derived from the amino acid sequence shown in SEQ ID NO:1. However, the invention also encompasses fragments of the variants of
30 the 39404 protein as described herein.

The invention thus also includes polypeptide fragments of the 38911 protein. Fragments can be derived from the amino acid sequence shown in SEQ

ID NO:3. However, the invention also encompasses fragments of the variants of the 38911 protein as described herein.

The invention thus also includes polypeptide fragments of the 26904 protein. Fragments can be derived from the amino acid sequence shown in SEQ

5 ID NO:5. However, the invention also encompasses fragments of the variants of the 26904 protein as described herein.

The fragments *per se* to which the invention pertains, however, are not to be construed as encompassing fragments that may be disclosed prior to the present invention (known fragments are encompassed in uses and methods specific for
10 tissues or disorders with which the gene is associated).

Fragments can retain one or more of the biological activities of the protein, for example, the ability to bind to a G-protein or ligand, as well as fragments that can be used as an immunogen to generate antibodies.

Biologically active fragments of the 39404 protein (peptides which are, for
15 example, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-100, or more amino acids in length) can comprise a domain or motif, e.g., an extracellular or intracellular domain or loop, one or more transmembrane segments or parts thereof, G-protein binding site, GPCR signature, glycosylation site, or phosphorylation site. In one embodiment, fragments are greater than eleven amino acids.

20 Such domains or motifs can be identified by means of routine computerized homology searching procedures.

Possible fragments include, but are not limited to: 1) soluble peptides comprising the entire amino terminal extracellular domain or parts thereof; 2) peptides comprising the entire carboxy terminal intracellular domain or parts
25 thereof; 3) peptides comprising the region spanning the entire transmembrane domain or parts thereof; 4) any of the specific transmembrane segments, or parts thereof; 5) any of the three intracellular or three extracellular loops, or parts thereof. Fragments further include combinations of the above fragments, such as an amino terminal domain combined with one or more transmembrane segments
30 and the attendant extra or intracellular loops or one or more transmembrane segments, and the attendant intra or extracellular loops, plus the carboxy terminal domain. Thus, any of the above fragments can be combined. Other fragments

include the mature protein from about amino acid 6 to the last amino acid. Other fragments contain the various functional sites described herein, such as phosphorylation sites or glycosylation sites, and a sequence containing the GPCR signature sequence. Fragments, for example, can extend in one or both directions from the functional site to encompass 5, 10, 15, 20, 30, 40, 50, or up to 100 amino acids. Further, fragments can include sub-fragments of the specific domains mentioned above, which sub-fragments retain the function of the domain from which they are derived. Fragments also include but are not limited to amino acid sequences greater than 5 amino acids, except for SILTLT (SEQ ID NO:7), SILFLTC (SEQ ID NO:8), or NLYSSILFLTC (SEQ ID NO:9) (however, it is understood that with regard to uses and methods of the invention, even these fragments and any other fragments that may be known prior to the invention are encompassed). In no way however are such fragments to be construed as encompassing fragments that may be found in the art, except as above indicated.

These regions can be identified by well-known methods involving computerized homology analysis.

Fragments also include antigenic fragments and specifically in regions shown to have a high antigenic index in Figure 3.

Accordingly, possible fragments include fragments defining a ligand-binding site, fragments defining a glycosylation site, fragments defining membrane association, fragments defining phosphorylation sites, and fragments defining interaction with G proteins and signal transduction. By this is intended a discrete fragment that provides the relevant function or allows the relevant function to be identified. In a preferred embodiment, the fragment contains the ligand-binding site.

Biologically active fragments of 38911 protein (peptides which are, for example, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-100, or more amino acids in length) can comprise a domain or motif, e.g., an extracellular or intracellular domain or loop, one or more transmembrane segments, or parts thereof, G-protein binding site, glycosylation sites, and cAMP- and cGMP-dependent, protein kinase C, and casein kinase II phosphorylation sites, and N-myristoylation sites.

Such domains or motifs can be identified by means of routine computerized homology searching procedures.

Possible fragments include, but are not limited to: 1) soluble peptides comprising the entire amino terminal extracellular domain about amino acid 1 to about amino acid 40 of SEQ ID NO:3, or parts thereof; 2) peptides comprising the entire carboxy terminal intracellular domain from about amino acid 259 to amino acid 337 of SEQ ID NO:3, or parts thereof; 3) peptides comprising the region spanning the entire transmembrane domain from about amino acid 41 to about amino acid 294, or parts thereof; 4) any of the specific transmembrane segments, or parts thereof; 5) any of the three intracellular or three extracellular loops, or parts thereof. Fragments further include combinations of the above fragments, such as an amino terminal domain combined with one or more transmembrane segments and the attendant extra or intracellular loops or one or more transmembrane segments, and the attendant intra or extracellular loops, plus the carboxy terminal domain. Thus, any of the above fragments can be combined. Other fragments include the mature protein from about amino acid 6 to 337. Other fragments contain the various functional sites described herein, such as phosphorylation sites, glycosylation sites, or myristoylation sites. Fragments, for example, can extend in one or both directions from the functional site to encompass 5, 10, 15, 20, 30, 40, 50, or up to 100 amino acids. Further, fragments can include sub-fragments of the specific domains mentioned above, which sub-fragments retain the function of the domain from which they are derived.

These regions can be identified by well-known methods involving computerized homology analysis.

Fragments also include amino acid sequences greater than 5 amino acids except for LAVADLL (SEQ ID NO:10), LALLLT (SEQ ID NO:11), LRRSLP (SEQ ID NO:12), FLVGDPGNA (SEQ ID NO:13), GNAMV (SEQ ID NO:14), LAVAD (SEQ ID NO:15), FLVGVPGNA (SEQ ID NO:16), ALLLT (SEQ ID NO:17), and ADLLCCLSLP (SEQ ID NO:18) (it is understood however that these fragments and any others that may have been disclosed prior to the invention may be encompassed in specific uses and methods disclosed herein relating to tissues/disorders with which the expression is associated). In no way however are

such fragments to be construed as encompassing fragments that may be found in the art except as just indicated.

Fragments also include antigenic fragments and specifically from regions shown to have a high antigenic index in Figure 9.

5 Accordingly, possible fragments include fragments defining a ligand-binding site, fragments defining a glycosylation site, fragments defining membrane association, fragments defining a phosphorylation site, fragments defining interaction with G proteins and signal transduction, and fragments defining a myristoylation site. By this is intended a discrete fragment that
10 provides the relevant function or allows the relevant function to be identified. In a preferred embodiment, the fragment contains the ligand-binding site.

Biologically active fragments of the 26904 protein (peptides which are, for example, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-100, or more amino acids in length) can comprise a domain or motif, e.g., an extracellular or intracellular
15 domain or loop, one or more transmembrane segments, or parts thereof, G-protein binding site, glycosylation site, cAMP, cGMP, protein kinase C, and casein kinase II phosphorylation site, N-myristoylation site, amidation, or ATP/GTP binding site.

Such domains or motifs can be identified by means of routine
20 computerized homology searching procedures.

Possible fragments include, but are not limited to: 1) soluble peptides comprising the entire amino terminal extracellular domain, or parts thereof; 2) peptides comprising the entire carboxy terminal intracellular domain, or parts thereof; 3) peptides comprising the region spanning the entire transmembrane
25 domain, or parts thereof; 4) any of the specific transmembrane segments, or parts thereof; 5) any of the three intracellular or three extracellular loops, or parts thereof. Fragments further include combinations of the above fragments, such as an amino terminal domain combined with one or more transmembrane segments and the attendant extra or intracellular loops or one or more transmembrane
30 segments, and the attendant intra or extracellular loops, plus the carboxy terminal domain. Thus, any of the above fragments can be combined. Other fragments include the mature protein from about amino acid 6 to 450. Other fragments

contain the various functional sites described herein, such as phosphorylation sites, glycosylation site, or myristoylation sites. Fragments, for example, can extend in one or both directions from the functional site to encompass 5, 10, 15, 20, 30, 40, 50, or up to 100 amino acids. Further, fragments can include sub-fragments of the specific domains mentioned above, which sub-fragments retain the function of the domain from which they are derived.

These regions can be identified by well-known methods involving computerized homology analysis.

Fragments also include amino acid sequences greater than four amino acids except for YVGAAHG (SEQ ID NO:19), LVHWCHGAPGVI (SEQ ID NO:20), QAYKVF (SEQ ID NO:21), EEKYL (SEQ ID NO:22), SLFEGMAG (SEQ ID NO:23), RFPAFEL (SEQ ID NO:24), LLQQME (SEQ ID NO:25), TFLCGDAGPLAV (SEQ ID NO:26), AGIYY (SEQ ID NO:27), SGNYP (SEQ ID NO:28), QAYKVFKEE (SEQ ID NO:29), DVIWQ (SEQ ID NO:30), KYLYRACKFAEWCLDYG (SEQ ID NO:31), ELLYGR (SEQ ID NO:32), PYSLFEG (SEQ ID NO:33), and VTFLCG (SEQ ID NO:34) (it is understood however that these fragments and any others that may have been disclosed prior to the invention are in fact encompassed by the invention in methods and uses disclosed herein relevant to specific tissues or disorders with which the gene is associated). In no way however are such fragments to be construed as encompassing fragments that may be found in the art, except as just indicated.

Fragments also include antigenic fragments and specifically from sites shown to have a high antigenic index in Figure 15.

Accordingly, possible fragments include but are not limited to fragments defining a ligand-binding site, fragments defining a glycosylation site, fragments defining membrane association, fragments defining phosphorylation sites, fragments defining interaction with G proteins and signal transduction, and fragments defining myristoylation sites. By this is intended a discrete fragment that provides the relevant function or allows the relevant function to be identified. In a preferred embodiment, the fragment contains the ligand-binding site.

The invention also provides 39404 protein fragments with immunogenic properties. These contain an epitope-bearing portion of the 39404 protein and

variants. The invention also provides 38911 protein fragments with immunogenic properties. These contain an epitope-bearing portion of the 38911 protein and variants. The invention also provides 26904 protein fragments with immunogenic properties. These contain an epitope-bearing portion of the 26904 protein and
5 variants. These peptides can contain at least 5-10, 11, 12, 13, at least 14, or between at least about 15 to about 30 amino acids.

Non-limiting examples of antigenic polypeptides that can be used to generate antibodies include peptides derived from the amino terminal extracellular domain or any of the extracellular loops. Regions having a high antigenicity index
10 are shown in Figures 3, 9, and 15.

The epitope-bearing receptor and polypeptides may be produced by any conventional means (Houghten, R.A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985)). Simultaneous multiple peptide synthesis is described in U.S. Patent No. 4,631,211.

15 Fragments can be discrete (not fused to other amino acids or polypeptides) or can be within a larger polypeptide. Further, several fragments can be comprised within a single larger polypeptide. In one embodiment a fragment designed for expression in a host can have heterologous pre- and pro-polypeptide regions fused to the amino terminus of the fragment and an additional region fused to the
20 carboxyl terminus of the fragment.

The invention thus provides chimeric or fusion proteins. These comprise a protein of the invention operatively linked to a heterologous protein having an amino acid sequence not substantially homologous to the protein. "Operatively linked" indicates that the protein of the invention and the heterologous protein are
25 fused in-frame. The heterologous protein can be fused to the N-terminus or C-terminus of the protein of the invention.

In one embodiment the fusion protein does not affect protein function *per se*. For example, the fusion protein can be a GST-fusion protein in which the sequences of the invention are fused to the C-terminus of the GST sequences.
30 Other types of fusion proteins include, but are not limited to, enzymatic fusion proteins, for example beta-galactosidase fusions, yeast two-hybrid GAL-4 fusions, poly-His fusions and Ig fusions. Such fusion proteins, particularly poly-His

fusions, can facilitate the purification of recombinant protein of the invention. In certain host cells (e.g., mammalian host cells), expression and/or secretion of a protein can be increased by using a heterologous signal sequence. Therefore, in another embodiment, the fusion protein contains a heterologous signal sequence at its C- or N-terminus.

EP-A-O 464 533 discloses fusion proteins comprising various portions of immunoglobulin constant regions. The Fc is useful in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). In drug discovery, for example, human proteins have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists. Bennett *et al.* (*J. Mol. Recog.* 8:52-58 (1995)) and Johanson *et al.* (*J. Biol. Chem.* 270, 16:9459-9471 (1995)). Thus, this invention also encompasses soluble fusion proteins containing a polypeptide of the invention and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclass (IgG, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. For some uses it is desirable to remove the Fc after the fusion protein has been used for its intended purpose, for example when the fusion protein is to be used as antigen for immunizations. In a particular embodiment, the Fc part can be removed in a simple way by a cleavage sequence which is also incorporated and can be cleaved with factor Xa.

A chimeric or fusion protein can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different protein sequences are ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and re-amplified to generate a chimeric gene sequence (see Ausubel *et al.*, *Current Protocols in Molecular Biology*, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST protein). A protein-encoding

nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein.

Another form of fusion protein is one that directly affects protein functions. Accordingly, a polypeptide is encompassed by the present invention in which one or more of the receptor domains (or parts thereof) has been replaced by homologous domains (or parts thereof) from another seven-transmembrane protein, for example another G-protein coupled receptor or other type of receptor. Accordingly, various permutations are possible. The amino terminal extracellular domain, or subregion thereof, (for example, ligand-binding) can be replaced with the domain or subregion from another ligand-binding receptor protein. Alternatively, the entire transmembrane domain, or any of the seven segments or loops, or parts thereof, for example, G-protein-binding/signal transduction, can be replaced. Finally, the carboxy terminal intracellular domain or subregion can be replaced. Thus, chimeric seven-transmembrane proteins/receptors can be formed in which one or more of the native domains or subregions has been replaced.

The isolated 39404 protein can be purified from cells that naturally express it, such as from breast, brain, kidney, vein, fetal kidney and fetal liver, shown in Figure 5, as well as aortic intimal proliferations and internal mammary artery as shown in Figures 6 and 7, purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods.

The isolated 38911 protein can be purified from cells that naturally express it, such as from those tissues shown in Figures 12 and 13, and especially osteoclasts, spleen, tonsils, liver, kidney, and testis, purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods.

The isolated 26904 protein can be purified from cells that naturally express it, such as from brain, purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods.

In one embodiment, the protein is produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the polypeptide is cloned into an expression vector, the expression vector introduced into a host cell and the protein expressed in the host cell. The protein can then be isolated from

the cells by an appropriate purification scheme using standard protein purification techniques.

Polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally-occurring amino acids. Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques well known in the art. Common modifications that occur naturally in polypeptides are described in basic texts, detailed monographs, and the research literature, and they are well known to those of skill in the art.

Accordingly, the polypeptides also encompass derivatives or analogs in which a substituted amino acid residue is not one encoded by the genetic code, in which a substituent group is included, in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence for purification of the mature polypeptide or a pro-protein sequence.

Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well-known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for

instance, are described in most basic texts, such as *Proteins - Structure and Molecular Properties*, 2nd Ed., T.E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as by Wold, F., *Posttranslational Covalent Modification of Proteins*, B.C. Johnson, Ed.,
5 Academic Press, New York 1-12 (1983); Seifter *et al.* (*Meth. Enzymol.* 182: 626-646 (1990)) and Rattan *et al.* *Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

As is also well known, polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of post-translation
10 events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by synthetic methods.

Modifications can occur anywhere in a polypeptide, including the peptide
15 backbone, the amino acid side-chains and the amino or carboxyl termini. Blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally-occurring and synthetic polypeptides. For instance, the amino terminal residue of polypeptides made in *E. coli*, prior to proteolytic processing, almost invariably will be N-formylmethionine.

20 The modifications can be a function of how the protein is made. For recombinant polypeptides, for example, the modifications will be determined by the host cell posttranslational modification capacity and the modification signals in the polypeptide amino acid sequence. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic
25 cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to efficiently express mammalian proteins having native patterns of glycosylation. Similar considerations apply to other modifications.

The same type of modification may be present in the same or varying
30 degree at several sites in a given polypeptide. Also, a given polypeptide may contain more than one type of modification.

Polypeptide uses

The polypeptides are useful for various biological assays as described in detail below. Since the 39404 gene is expressed in the tissues shown in Figures 5-7, the assays are particularly useful in cells derived from these tissue types, and particularly the tissues in which the gene is highly expressed, such as brain, kidney, fetal kidney, fetal liver, internal mammary artery, and aortic intimal proliferations. Furthermore, since the gene is expressed in these tissues, assays involving the protein in pathological tissue/disorders, particularly applies to disorders involving these tissues and especially the tissues in which the gene is highly expressed. Moreover, since the gene is expressed in aortic intimal proliferations (atheroplaques), and heart tissue from patients with congestive heart failure, ischemia, and myopathy, the assays and methods involving pathology/disorders are particularly relevant in these disorders.

Since the 38911 gene is expressed in the tissues shown in Figures 12 and 13, the assays are particularly useful in cells derived from these tissue types, and particularly the cells and tissues in which the gene is highly expressed, such as spleen, tonsils, kidney, testis, liver, and osteoclasts. Furthermore, since the gene is expressed in these tissues, assays involving the protein in pathological tissue/disorders, particularly applies to disorders involving these tissues and especially the tissues in which the gene is highly expressed. Since the gene is highly expressed in osteoclasts, assays and methods involving pathology/disorders are particularly relevant to disorders involving osteoclast function. These disorders include but are not limited to those involved in bone growth and development, particularly disorders involving bone mass, such as osteoporosis. In addition, since relatively high expression occurs in fibrotic livers, liver fibrosis is a disorder relevant to expression of the 38911 receptor.

Further, expression of the 38911 receptor is relevant to inflammation, in view of homology to the C5a receptor.

Disorders involving the spleen include, but are not limited to, splenomegaly, including nonspecific acute splenitis, congestive splenomegaly, and splenic infarcts; neoplasms, congenital anomalies, and rupture. Disorders associated with splenomegaly include infections, such as nonspecific splenitis,

infectious mononucleosis, tuberculosis, typhoid fever, brucellosis, cytomegalovirus, syphilis, malaria, histoplasmosis, toxoplasmosis, kala-azar, trypanosomiasis, schistosomiasis, leishmaniasis, and echinococcosis; congestive states related to partial hypertension, such as cirrhosis of the liver, portal or splenic vein thrombosis, and cardiac failure; lymphohematogenous disorders, such as Hodgkin disease, non-Hodgkin lymphomas/leukemia, multiple myeloma, myeloproliferative disorders, hemolytic anemias, and thrombocytopenic purpura; immunologic-inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus; storage diseases such as Gaucher disease, Niemann-Pick disease, and mucopolysaccharidoses; and other conditions, such as amyloidosis, primary neoplasms and cysts, and secondary neoplasms.

Disorders involving the lung include, but are not limited to, congenital anomalies; atelectasis; diseases of vascular origin, such as pulmonary congestion and edema, including hemodynamic pulmonary edema and edema caused by microvascular injury, adult respiratory distress syndrome (diffuse alveolar damage), pulmonary embolism, hemorrhage, and infarction, and pulmonary hypertension and vascular sclerosis; chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis; diffuse interstitial (infiltrative, restrictive) diseases, such as pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia (pulmonary infiltration with eosinophilia), *Bronchiolitis obliterans*-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, including Goodpasture syndrome, idiopathic pulmonary hemosiderosis and other hemorrhagic syndromes, pulmonary involvement in collagen vascular disorders, and pulmonary alveolar proteinosis; complications of therapies, such as drug-induced lung disease, radiation-induced lung disease, and lung transplantation; tumors, such as bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

Disorders involving the colon include, but are not limited to, congenital anomalies, such as atresia and stenosis, Meckel diverticulum, congenital aganglionic megacolon-Hirschsprung disease; enterocolitis, such as diarrhea and dysentery, infectious enterocolitis, including viral gastroenteritis, bacterial enterocolitis, necrotizing enterocolitis, antibiotic-associated colitis (pseudomembranous colitis), and collagenous and lymphocytic colitis, miscellaneous intestinal inflammatory disorders, including parasites and protozoa, acquired immunodeficiency syndrome, transplantation, drug-induced intestinal injury, radiation enterocolitis, neutropenic colitis (typhlitis), and diversion colitis; idiopathic inflammatory bowel disease, such as Crohn disease and ulcerative colitis; tumors of the colon, such as non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

Disorders involving the liver include, but are not limited to, hepatic injury; jaundice and cholestasis, such as bilirubin and bile formation; hepatic failure and cirrhosis, such as cirrhosis, portal hypertension, including ascites, portosystemic shunts, and splenomegaly; infectious disorders, such as viral hepatitis, including hepatitis A-E infection and infection by other hepatitis viruses, clinicopathologic syndromes, such as the carrier state, asymptomatic infection, acute viral hepatitis, chronic viral hepatitis, and fulminant hepatitis; autoimmune hepatitis; drug- and toxin-induced liver disease, such as alcoholic liver disease; inborn errors of metabolism and pediatric liver disease, such as hemochromatosis, Wilson disease, α_1 -antitrypsin deficiency, and neonatal hepatitis; intrahepatic biliary tract disease, such as secondary biliary cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, and anomalies of the biliary tree; circulatory disorders, such as impaired blood flow into the liver, including hepatic artery compromise and portal vein obstruction and thrombosis, impaired blood flow through the liver, including passive congestion and centrilobular necrosis and peliosis hepatis, hepatic vein outflow obstruction, including hepatic vein thrombosis (Budd-Chiari syndrome) and veno-occlusive disease; hepatic disease associated with pregnancy, such as preeclampsia and eclampsia, acute fatty liver of pregnancy, and intrahepatic cholestasis of pregnancy; hepatic complications of organ or bone marrow transplantation, such as drug toxicity after bone marrow transplantation, graft-

versus-host disease and liver rejection, and nonimmunologic damage to liver allografts; tumors and tumorous conditions, such as nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

- 5 Disorders involving the uterus and endometrium include, but are not limited to, endometrial histology in the menstrual cycle; functional endometrial disorders, such as anovulatory cycle, inadequate luteal phase, oral contraceptives and induced endometrial changes, and menopausal and postmenopausal changes; inflammations, such as chronic endometritis; adenomyosis; endometriosis;
- 10 endometrial polyps; endometrial hyperplasia; malignant tumors, such as carcinoma of the endometrium; mixed Müllerian and mesenchymal tumors, such as malignant mixed Müllerian tumors; tumors of the myometrium, including leiomyomas, leiomyosarcomas, and endometrial stromal tumors.

- Disorders involving the brain include, but are not limited to, disorders
- 15 involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury;
- 20 cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and
- 25 vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis,
- 30 including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex virus Type 1*, *Herpes simplex virus Type 2*,

- Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal
- 5 leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic
- 10 encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy,
- 15 including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy
- 20 (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic
- 25 diseases, including vitamin deficiencies such as thiamine (vitamin B₁) deficiency and vitamin B₁₂ deficiency, neurologic sequelae of metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas,
- 30 including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and ependymoma and related paraventricular

mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromatosis; including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

Disorders involving T-cells include, but are not limited to, cell-mediated hypersensitivity, such as delayed type hypersensitivity and T-cell-mediated cytotoxicity, and transplant rejection; autoimmune diseases, such as systemic lupus erythematosus, Sjögren syndrome, systemic sclerosis, inflammatory myopathies, mixed connective tissue disease, and polyarteritis nodosa and other vasculitides; immunologic deficiency syndromes, including but not limited to, primary immunodeficiencies, such as thymic hypoplasia, severe combined immunodeficiency diseases, and AIDS; leukopenia; reactive (inflammatory) proliferations of white cells, including but not limited to, leukocytosis, acute nonspecific lymphadenitis, and chronic nonspecific lymphadenitis; neoplastic proliferations of white cells, including but not limited to lymphoid neoplasms, such as precursor T-cell neoplasms, such as acute lymphoblastic leukemia/lymphoma, peripheral T-cell and natural killer cell neoplasms that include peripheral T-cell lymphoma, unspecified, adult T-cell leukemia/lymphoma, mycosis fungoides and Sézary syndrome, and Hodgkin disease.

Diseases of the skin, include but are not limited to, disorders of pigmentation and melanocytes, including but not limited to, vitiligo, freckle, melasma, lentigo, nevocellular nevus, dysplastic nevi, and malignant melanoma; benign epithelial tumors, including but not limited to, seborrheic keratoses, acanthosis nigricans, fibroepithelial polyp, epithelial cyst, keratoacanthoma, and adnexal (appendage) tumors; premalignant and malignant epidermal tumors, including but not limited to, actinic keratosis, squamous cell carcinoma, basal cell

carcinoma, and merkel cell carcinoma; tumors of the dermis, including but not limited to, benign fibrous histiocytoma, dermatofibrosarcoma protuberans, xanthomas, and dermal vascular tumors; tumors of cellular immigrants to the skin, including but not limited to, histiocytosis X, mycosis fungoides (cutaneous T-cell lymphoma), and mastocytosis; disorders of epidermal maturation, including but not limited to, ichthyosis; acute inflammatory dermatoses, including but not limited to, urticaria, acute eczematous dermatitis, and erythema multiforme; chronic inflammatory dermatoses, including but not limited to, psoriasis, lichen planus, and lupus erythematosus; blistering (bullous) diseases, including but not limited to, pemphigus, bullous pemphigoid, dermatitis herpetiformis, and noninflammatory blistering diseases: epidermolysis bullosa and porphyria; disorders of epidermal appendages, including but not limited to, acne vulgaris; panniculitis, including but not limited to, erythema nodosum and erythema induratum; and infection and infestation, such as verrucae, molluscum contagiosum, impetigo, superficial fungal infections, and arthropod bites, stings, and infestations.

In normal bone marrow, the myelocytic series (polymorphoneuclear cells) make up approximately 60% of the cellular elements, and the erythrocytic series, 20-30%. Lymphocytes, monocytes, reticular cells, plasma cells and megakaryocytes together constitute 10-20%. Lymphocytes make up 5-15% of normal adult marrow. In the bone marrow, cell types are add mixed so that precursors of red blood cells (erythroblasts), macrophages (monoblasts), platelets (megakaryocytes), polymorphoneuclear leucocytes (myeloblasts), and lymphocytes (lymphoblasts) can be visible in one microscopic field. In addition, stem cells exist for the different cell lineages, as well as a precursor stem cell for the committed progenitor cells of the different lineages. The various types of cells and stages of each would be known to the person of ordinary skill in the art and are found, for example, on page 42 (Figure 2-8) of *Immunology, Immunopathology and Immunity*, Fifth Edition, Sell *et al.* Simon and Schuster (1996), incorporated by reference for its teaching of cell types found in the bone marrow. According, the invention is directed to disorders arising from these cells. These disorders include but are not limited to the following: diseases involving hematopoietic stem

cells; committed lymphoid progenitor cells; lymphoid cells including B and T-cells; committed myeloid progenitors, including monocytes, granulocytes, and megakaryocytes; and committed erythroid progenitors. These include but are not limited to the leukemias, including B-lymphoid leukemias, T-lymphoid leukemias, undifferentiated leukemias; erythroleukemia, megakaryoblastic leukemia, monocytic leukemia with and without differentiation; chronic and acute lymphoblastic leukemia, chronic and acute lymphocytic leukemia, chronic and acute myelogenous leukemia, lymphoma, myelo dysplastic syndrome, chronic and acute myeloid leukemia, myelomonocytic leukemia; chronic and acute myeloblastic leukemia, chronic and acute myelogenous leukemia, chronic and acute promyelocytic leukemia, chronic and acute myelocytic leukemia, hematologic malignancies of monocyte-macrophage lineage, such as juvenile chronic myelogenous leukemia; secondary AML, antecedent hematological disorder; refractory anemia; aplastic anemia; reactive cutaneous angioendotheliomatosis; fibrosing disorders involving altered expression in dendritic cells, disorders including systemic sclerosis, E-M syndrome, epidemic toxic oil syndrome, eosinophilic fasciitis localized forms of scleroderma, keloid, and fibrosing colonopathy; angiomatoid malignant fibrous histiocytoma; carcinoma, including primary head and neck squamous cell carcinoma; sarcoma, including kaposi's sarcoma; fibroadenoma and phyllodes tumors, including mammary fibroadenoma; stromal tumors; phyllodes tumors, including histiocytoma; erythroblastosis; neurofibromatosis; diseases of the vascular endothelium; demyelinating, particularly in old lesions; gliosis, vasogenic edema, vascular disease, Alzheimer's and Parkinson's disease; T-cell lymphomas; B-cell lymphomas.

Disorders involving the heart, include but are not limited to, heart failure, including but not limited to, cardiac hypertrophy, left-sided heart failure, and right-sided heart failure; ischemic heart disease, including but not limited to angina pectoris, myocardial infarction, chronic ischemic heart disease, and sudden cardiac death; hypertensive heart disease, including but not limited to, systemic (left-sided) hypertensive heart disease and pulmonary (right-sided) hypertensive heart disease; valvular heart disease, including but not limited to, valvular degeneration

caused by calcification, such as calcific aortic stenosis, calcification of a congenitally bicuspid aortic valve, and mitral annular calcification, and myxomatous degeneration of the mitral valve (mitral valve prolapse), rheumatic fever and rheumatic heart disease, infective endocarditis, and noninfected
5 vegetations, such as nonbacterial thrombotic endocarditis and endocarditis of systemic lupus erythematosus (Libman-Sacks disease), carcinoid heart disease, and complications of artificial valves; myocardial disease, including but not limited to dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and myocarditis; pericardial disease, including but not limited to,
10 pericardial effusion and hemopericardium and pericarditis, including acute pericarditis and healed pericarditis, and rheumatoid heart disease; neoplastic heart disease, including but not limited to, primary cardiac tumors, such as myxoma, lipoma, papillary fibroelastoma, rhabdomyoma, and sarcoma, and cardiac effects of noncardiac neoplasms; congenital heart disease, including but not limited to,
15 left-to-right shunts--late cyanosis, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect, right-to-left shunts--early cyanosis, such as tetralogy of fallot, transposition of great arteries, truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection, obstructive congenital anomalies, such as coarctation of aorta,
20 pulmonary stenosis and atresia, and aortic stenosis and atresia, and disorders involving cardiac transplantation.

Disorders involving blood vessels include, but are not limited to, responses of vascular cell walls to injury, such as endothelial dysfunction and endothelial activation and intimal thickening; vascular diseases including, but not limited to,
25 congenital anomalies, such as arteriovenous fistula, atherosclerosis, and hypertensive vascular disease, such as hypertension; inflammatory disease--the vasculitides, such as giant cell (temporal) arteritis, Takayasu arteritis, polyarteritis nodosa (classic), Kawasaki syndrome (mucocutaneous lymph node syndrome), microscopic polyanglitis (microscopic polyarteritis, hypersensitivity or
30 leukocytoclastic angitis), Wegener granulomatosis, thromboanglitis obliterans (Buerger disease), vasculitis associated with other disorders, and infectious arteritis; Raynaud disease; aneurysms and dissection, such as abdominal aortic

aneurysms, syphilitic (luetic) aneurysms, and aortic dissection (dissecting hematoma); disorders of veins and lymphatics, such as varicose veins, thrombophlebitis and phlebothrombosis, obstruction of superior vena cava (superior vena cava syndrome), obstruction of inferior vena cava (inferior vena cava syndrome), and lymphangitis and lymphedema; tumors, including benign tumors and tumor-like conditions, such as hemangioma, lymphangioma, glomus tumor (glomangioma), vascular ectasias, and bacillary angiomatosis, and intermediate-grade (borderline low-grade malignant) tumors, such as Kaposi sarcoma and hemangioendothelioma, and malignant tumors, such as angiosarcoma and hemangiopericytoma; and pathology of therapeutic interventions in vascular disease, such as balloon angioplasty and related techniques and vascular replacement, such as coronary artery bypass graft surgery.

Disorders involving red cells include, but are not limited to, anemias, such as hemolytic anemias, including hereditary spherocytosis, hemolytic disease due to erythrocyte enzyme defects: glucose-6-phosphate dehydrogenase deficiency, sickle cell disease, thalassemia syndromes, paroxysmal nocturnal hemoglobinuria, immunohemolytic anemia, and hemolytic anemia resulting from trauma to red cells; and anemias of diminished erythropoiesis, including megaloblastic anemias, such as anemias of vitamin B12 deficiency: pernicious anemia, and anemia of folate deficiency, iron deficiency anemia, anemia of chronic disease, aplastic anemia, pure red cell aplasia, and other forms of marrow failure.

Disorders involving the thymus include developmental disorders, such as DiGeorge syndrome with thymic hypoplasia or aplasia; thymic cysts; thymic hypoplasia, which involves the appearance of lymphoid follicles within the thymus, creating thymic follicular hyperplasia; and thymomas, including germ cell tumors, lymphomas, Hodgkin disease, and carcinoids. Thymomas can include benign or encapsulated thymoma, and malignant thymoma Type I (invasive thymoma) or Type II, designated thymic carcinoma.

Disorders involving B-cells include, but are not limited to precursor B-cell neoplasms, such as lymphoblastic leukemia/lymphoma. Peripheral B-cell neoplasms include, but are not limited to, chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma,

Burkitt lymphoma, plasma cell neoplasms, multiple myeloma, and related entities, lymphoplasmacytic lymphoma (Waldenström macroglobulinemia), mantle cell lymphoma, marginal zone lymphoma (MALToma), and hairy cell leukemia.

- Disorders involving the kidney include, but are not limited to, congenital
- 5 anomalies including, but not limited to, cystic diseases of the kidney, that include but are not limited to, cystic renal dysplasia, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, and cystic diseases of renal medulla, which include, but are not limited to, medullary sponge kidney, and nephronophthisis-uremic medullary cystic disease
- 10 complex, acquired (dialysis-associated) cystic disease, such as simple cysts; glomerular diseases including pathologies of glomerular injury that include, but are not limited to, in situ immune complex deposition, that includes, but is not limited to, anti-GBM nephritis, Heymann nephritis, and antibodies against planted antigens, circulating immune complex nephritis, antibodies to glomerular cells,
- 15 cell-mediated immunity in glomerulonephritis, activation of alternative complement pathway, epithelial cell injury, and pathologies involving mediators of glomerular injury including cellular and soluble mediators, acute glomerulonephritis, such as acute proliferative (poststreptococcal, postinfectious) glomerulonephritis, including but not limited to, poststreptococcal
- 20 glomerulonephritis and nonstreptococcal acute glomerulonephritis, rapidly progressive (crescentic) glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis (membranous nephropathy), *minimal change disease* (lipoid nephrosis), focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, IgA nephropathy (Berger disease), focal proliferative and
- 25 necrotizing glomerulonephritis (focal glomerulonephritis), hereditary nephritis, including but not limited to, Alport syndrome and thin membrane disease (benign familial hematuria), chronic glomerulonephritis, glomerular lesions associated with systemic disease, including but not limited to, systemic lupus erythematosus, Henoch-Schönlein purpura, bacterial endocarditis, diabetic glomerulosclerosis,
- 30 amyloidosis, fibrillary and immunotactoid glomerulonephritis, and other systemic disorders; diseases affecting tubules and interstitium, including acute tubular necrosis and tubulointerstitial nephritis, including but not limited to, pyelonephritis

and urinary tract infection, acute pyelonephritis, chronic pyelonephritis and reflux nephropathy, and tubulointerstitial nephritis induced by drugs and toxins, including but not limited to, acute drug-induced interstitial nephritis, analgesic abuse nephropathy, nephropathy associated with nonsteroidal anti-inflammatory drugs, and other tubulointerstitial diseases including, but not limited to, urate nephropathy, hypercalcemia and nephrocalcinosis, and multiple myeloma; diseases of blood vessels including benign nephrosclerosis, malignant hypertension and accelerated nephrosclerosis, renal artery stenosis, and thrombotic microangiopathies including, but not limited to, classic (childhood) hemolytic-uremic syndrome, adult hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura, idiopathic HUS/TTP, and other vascular disorders including, but not limited to, atherosclerotic ischemic renal disease, atheroembolic renal disease, sickle cell disease nephropathy, diffuse cortical necrosis, and renal infarcts; urinary tract obstruction (obstructive uropathy); urolithiasis (renal calculi, stones); and tumors of the kidney including, but not limited to, benign tumors, such as renal papillary adenoma, renal fibroma or hamartoma (renomedullary interstitial cell tumor), angiomyolipoma, and oncocytoma, and malignant tumors, including renal cell carcinoma (hypernephroma, adenocarcinoma of kidney), which includes urothelial carcinomas of renal pelvis.

Disorders of the breast include, but are not limited to, disorders of development; inflammations, including but not limited to, acute mastitis, periductal mastitis, periductal mastitis (recurrent subareolar abscess, squamous metaplasia of lactiferous ducts), mammary duct ectasia, fat necrosis, granulomatous mastitis, and pathologies associated with silicone breast implants; fibrocystic changes; proliferative breast disease including, but not limited to, epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors including, but not limited to, stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, no special type, invasive lobular carcinoma, medullary carcinoma,

colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms.

Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

- 5 Disorders involving the testis and epididymis include, but are not limited to, congenital anomalies such as cryptorchidism, regressive changes such as atrophy, inflammations such as nonspecific epididymitis and orchitis, granulomatous (autoimmune) orchitis, and specific inflammations including, but not limited to, gonorrhea, mumps, tuberculosis, and syphilis, vascular disturbances
- 10 including torsion, testicular tumors including germ cell tumors that include, but are not limited to, seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumor choriocarcinoma, teratoma, and mixed tumors, tumor of sex cord-gonadal stroma including, but not limited to, leydig (interstitial) cell tumors and sertoli cell tumors (androblastoma), and testicular lymphoma, and miscellaneous
- 15 lesions of tunica vaginalis.

Disorders involving the prostate include, but are not limited to, inflammations, benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or hyperplasia), and tumors such as carcinoma.

- Disorders involving the thyroid include, but are not limited to,
- 20 hyperthyroidism; hypothyroidism including, but not limited to, cretinism and myxedema; thyroiditis including, but not limited to, hashimoto thyroiditis, subacute (granulomatous) thyroiditis, and subacute lymphocytic (painless) thyroiditis; Graves disease; diffuse and multinodular goiter including, but not limited to, diffuse nontoxic (simple) goiter and multinodular goiter; neoplasms of
- 25 the thyroid including, but not limited to, adenomas, other benign tumors, and carcinomas, which include, but are not limited to, papillary carcinoma, follicular carcinoma, medullary carcinoma, and anaplastic carcinoma; and congenital anomalies.

- Disorders involving the skeletal muscle include tumors such as
- 30 rhabdomyosarcoma.

Disorders involving the pancreas include those of the exocrine pancreas such as congenital anomalies, including but not limited to, ectopic pancreas;

pancreatitis, including but not limited to, acute pancreatitis; cysts, including but not limited to, pseudocysts; tumors, including but not limited to, cystic tumors and carcinoma of the pancreas; and disorders of the endocrine pancreas such as, diabetes mellitus; islet cell tumors, including but not limited to, insulinomas, gastrinomas, and other rare islet cell tumors.

Disorders involving the small intestine include the malabsorption syndromes such as, celiac sprue, tropical sprue (postinfectious sprue), whipple disease, disaccharidase (lactase) deficiency, abetalipoproteinemia, and tumors of the small intestine including adenomas and adenocarcinoma.

Disorders related to reduced platelet number, thrombocytopenia, include idiopathic thrombocytopenic purpura, including acute idiopathic thrombocytopenic purpura, drug-induced thrombocytopenia, HIV-associated thrombocytopenia, and thrombotic microangiopathies: thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome.

Disorders involving precursor T-cell neoplasms include precursor T lymphoblastic leukemia/lymphoma. Disorders involving peripheral T-cell and natural killer cell neoplasms include T-cell chronic lymphocytic leukemia, large granular lymphocytic leukemia, mycosis fungoides and Sézary syndrome, peripheral T-cell lymphoma, unspecified, angioimmunoblastic T-cell lymphoma, angiocentric lymphoma (NK/T-cell lymphoma^{4a}), intestinal T-cell lymphoma, adult T-cell leukemia/lymphoma, and anaplastic large cell lymphoma.

Disorders involving the ovary include, for example, polycystic ovarian disease, Stein-leventhal syndrome, Pseudomyxoma peritonei and stromal hyperthecosis; ovarian tumors such as, tumors of coelomic epithelium, serous tumors, mucinous tumors, endometrioid tumors, clear cell adenocarcinoma, cystadenofibroma, brenner tumor, surface epithelial tumors; germ cell tumors such as mature (benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stromal tumors such as, granulosa-theca cell tumors, thecoma-fibromas, androblastomas, Hill cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

Bone-forming cells include the osteoprogenitor cells, osteoblasts, and osteocytes. The disorders of the bone are complex because they may have an impact on the skeleton during any of its stages of development. Hence, the disorders may have variable manifestations and may involve one, multiple or all bones of the body. Such disorders include, congenital malformations, achondroplasia and thanatophoric dwarfism, diseases associated with abnormal matix such as type 1 collagen disease, osteoporosis, Paget's disease, rickets, osteomalacia, high-turnover osteodystrophy, low-turnover of aplastic disease, osteonecrosis, pyogenic osteomyelitis, tuberculous osteomyelitis, osteoma, osteoid osteoma, osteoblastoma, osteosarcoma, osteochondroma, chondromas, chondroblastoma, chondromyxoid fibroma, chondrosarcoma, fibrous cortical defects, fibrous dysplasia, fibrosarcoma, malignant fibrous histiocytoma, Ewing's sarcoma, primitive neuroectodermal tumor, giant cell tumor, and metastatic tumors.

The polypeptides of the invention are useful for producing antibodies specific for the 39404, 38911, and 26904 protein, regions, or fragments. Regions having a high antigenicity index score are shown in Figures 3, 9, and 15.

The polypeptides, variants, and fragments (including those which may have been disclosed prior to the present invention) are useful for biological assays related to seven-transmembrane proteins/GPCRs. Such assays involve any of the known seven-transmembrane protein/GPCR functions or activities or properties useful for diagnosis and treatment of seven-transmembrane protein/GPCR-related conditions.

The polypeptides of the invention are also useful in drug screening assays, in cell-based or cell-free systems. Cell-based systems can be native, i.e., cells that normally express the protein, as a biopsy or expanded in cell culture. In one embodiment, however, cell-based assays involve recombinant host cells expressing the protein.

Determining the ability of the test compound to interact with the polypeptide can also comprise determining the ability of the test compound to preferentially bind to the polypeptide as compared to the ability of the ligand, or a biologically active portion thereof, to bind to the polypeptide.

The polypeptides can be used to identify compounds that modulate protein activity. Such compounds, for example, can increase or decrease affinity or rate of binding to a known ligand, compete with ligand for binding to the protein, or displace ligand bound to the protein. 39404, 38911, and 26904 protein and
5 appropriate variants and fragments can be used in high-throughput screens to assay candidate compounds for the ability to bind to the protein. These compounds can be further screened against a functional protein to determine the effect of the compound on the protein activity. Compounds can be identified that activate (agonist) or inactivate (antagonist) the protein to a desired degree. Modulatory
10 methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). Examples for the 39404 protein include but are not limited to purine analogs such as those discussed above. Examples for the 38911 protein include but are not limited to C5a and C5a analogs.

15 The polypeptides of the invention can be used to screen a compound for the ability to stimulate or inhibit interaction between the protein and a target molecule that normally interacts with the protein. The target can be ligand or a component of the signal pathway with which the protein normally interacts (for example, a G-protein or other interactor involved in cAMP or phosphatidylinositol
20 turnover and/or adenylate cyclase, or phospholipase C activation). The assay includes the steps of combining the protein with a candidate compound under conditions that allow the protein or fragment to interact with the target molecule, and to detect the formation of a complex between the protein and the target or to detect the biochemical consequence of the interaction with the protein and the
25 target, such as any of the associated effects of signal transduction such as G-protein phosphorylation, cyclic AMP or phosphatidylinositol turnover, and adenylate cyclase or phospholipase C activation.

Determining the ability of the protein to bind to a target molecule can also be accomplished using a technology such as real-time Bimolecular Interaction
30 Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without

labeling any of the interactants (e.g., BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233. Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 97:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra*).

Candidate compounds include, for example, 1) purine analogs (39404), 2) peptides such as soluble peptides, including C5a (38911), C5a fragments, and derivatives thereof, Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam *et al.*, *Nature* 354:82-84 (1991); Houghten *et al.*, *Nature* 354:84-86 (1991)) and combinatorial chemistry-derived molecular libraries made of D- and/or L- configuration amino acids; 3) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g.,

Songyang *et al.*, *Cell* 72:767-778 (1993)); 4) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies); and 5) small organic and inorganic molecules (e.g.,
5 molecules obtained from combinatorial and natural product libraries).

One candidate compound is a soluble full-length protein or fragment that competes for ligand binding. Other candidate compounds include mutant proteins or appropriate fragments containing mutations that affect protein function and thus compete for ligand. Accordingly, a fragment that competes for ligand, for
10 example with a higher affinity, or a fragment that binds ligand but does not allow release, is encompassed by the invention.

The invention provides other end points to identify compounds that modulate (stimulate or inhibit) receptor activity. The assays typically involve an assay of events in the signal transduction pathway that indicate receptor activity.
15 Thus, the expression of genes that are up- or down-regulated in response to the receptor protein dependent signal cascade can be assayed. In one embodiment, the regulatory region of such genes can be operably linked to a marker that is easily detectable, such as luciferase. Alternatively, phosphorylation of the protein, or a protein target, could also be measured.

20 Binding and/or activating compounds can also be screened by using chimeric proteins in which the amino terminal extracellular domain, or parts thereof, the entire transmembrane domain or subregions, such as any of the seven transmembrane segments or any of the intracellular or extracellular loops and the carboxy terminal intracellular domain, or parts thereof, can be replaced by
25 heterologous domains or subregions. For example, a G-protein-binding region can be used that interacts with a different G-protein than that which is recognized by the native receptor. Accordingly, a different set of signal transduction components is available as an end-point assay for activation. Alternatively, the entire transmembrane portion or subregions (such as transmembrane segments or
30 intracellular or extracellular loops) can be replaced with the entire transmembrane portion or subregions specific to a host cell that is different from the host cell from which the amino terminal extracellular domain and/or the G-protein-binding

region are derived. This allows for assays to be performed in other than the specific host cell from which the protein is derived. Alternatively, the amino terminal extracellular domain (and/or other ligand-binding regions) could be replaced by a domain (and/or other binding region) binding a different ligand, thus, providing an assay for test compounds that interact with the heterologous amino terminal extracellular domain (or region) but still cause signal transduction. Finally, activation can be detected by a reporter gene containing an easily detectable coding region operably linked to a transcriptional regulatory sequence that is part of the native signal transduction pathway.

10 The polypeptides of the invention are also useful in competition binding assays in methods designed to discover compounds that interact with the protein. Thus, a compound is exposed to a polypeptide of the invention under conditions that allow the compound to bind or to otherwise interact with the polypeptide. Soluble polypeptide is also added to the mixture. If the test compound interacts with the soluble polypeptide, it decreases the amount of complex formed or activity from the protein target. This type of assay is particularly useful in cases in which compounds are sought that interact with specific regions of the protein. Thus, the soluble polypeptide that competes with the target region is designed to contain peptide sequences corresponding to the region of interest.

20 To perform cell free drug screening assays, it is desirable to immobilize either the protein, or fragment, or its target molecule to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay.

Techniques for immobilizing proteins on matrices can be used in the drug screening assays. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, glutathione-S-transferase/39404, 38911, and 26904 fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the cell lysates (e.g., ³⁵S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads are washed to

remove any unbound label, and the matrix immobilized and radiolabel determined directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of receptor-binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques. For example, either the polypeptide or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin using techniques well known in the art. Alternatively, antibodies reactive with the protein but which do not interfere with binding of the protein to its target molecule can be derivatized to the wells of the plate, and the protein trapped in the wells by antibody conjugation. Preparations of a protein of the invention-binding protein and a candidate compound are incubated in the protein of the invention-presenting wells and the amount of complex trapped in the well can be quantitated. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the protein target molecule, or which are reactive with protein and compete with the target molecule; as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

Modulators of 39404 protein activity identified according to these drug screening assays can be used to treat a subject with a disorder mediated by the protein pathway, by treating cells that express the 39404 protein, such as in breast, brain, kidney, vein, fetal kidney, fetal liver, aortic intimal proliferations, internal mammary artery, and cells involved in congestive heart failure, ischemia, and myopathy, for example, cardiomyocytes. Modulators of 38911 protein activity identified according to these drug screening assays can be used to treat a subject with a disorder mediated by the protein pathway, by treating cells that express the 38911 protein, such as in Figures 12 and 13, and especially osteoclasts, liver, kidney, and testis. Modulators of 39604 protein activity identified according to these drug screening assays can be used to treat a subject with a disorder mediated by the protein pathway, by treating cells that express the 26904 protein, such as in brain.

Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate,
5 relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

A therapeutic agent or compound includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

The polypeptides of the invention are thus useful for treating a protein of
10 the invention-associated disorder characterized by aberrant expression or activity of a protein of the invention. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) expression or activity of the protein. In another embodiment, the
15 method involves administering a protein as therapy to compensate for reduced or aberrant expression or activity of the protein.

Stimulation of protein activity is desirable in situations in which the protein is abnormally downregulated and/or in which increased protein activity is likely to have a beneficial effect. Likewise, inhibition of protein activity is
20 desirable in situations in which the protein is abnormally upregulated and/or in which decreased protein activity is likely to have a beneficial effect. In one example of such a situation, a subject has a disorder characterized by aberrant development or cellular differentiation. In another example of such a situation, the subject has a proliferative disease (e.g., cancer) or a disorder characterized by an
25 aberrant hematopoietic response. In another example of such a situation, it is desirable to achieve tissue regeneration in a subject (e.g., where a subject has undergone brain or spinal cord injury and it is desirable to regenerate neuronal tissue in a regulated manner).

In yet another aspect of the invention, the proteins of the invention can be
30 used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924;

Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO 94/10300), to identify other proteins (captured proteins) which bind to or interact with the proteins of the invention and modulate their activity.

5 The 39404 polypeptides also are useful to provide a target for diagnosing a disease or predisposition to disease mediated by the protein, especially in breast, brain, kidney, vein, fetal kidney, fetal liver, aortic intimal proliferations, internal mammary artery, and especially in congestive heart failure, ischemia, and myopathy. Disorders, however, also include diseases of other tissues in which the gene is expressed as shown in Figures 5-7. Tissue disorders are described in more
10 detail hereinabove. The 38911 polypeptides also are useful to provide a target for diagnosing a disease or predisposition to disease mediated by the protein, especially in osteoclasts, liver, kidney, and testis. The 26904 polypeptides also are useful to provide a target for diagnosing a disease or predisposition to disease mediated by the protein, such as in brain. Accordingly, methods are provided for
15 detecting the presence, or levels of, the protein in a cell, tissue, or organism. The method involves contacting a biological sample with a compound capable of interacting with the protein such that the interaction can be detected.

One agent for detecting a protein of the invention is an antibody capable of selectively binding to the protein. A biological sample includes tissues, cells and
20 biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

The proteins of the invention also provide a target for diagnosing active disease, or predisposition to disease, in a patient having a variant protein. Thus, a protein of the invention can be isolated from a biological sample, assayed for the
25 presence of a genetic mutation that results in an aberrant protein. This includes amino acid substitution, deletion, insertion, rearrangement, (as the result of aberrant splicing events), and inappropriate post-translational modification. Analytic methods include altered electrophoretic mobility, altered tryptic peptide digest, altered protein activity in cell-based or cell-free assay, alteration in ligand
30 or antibody-binding pattern, altered isoelectric point, direct amino acid sequencing, and any other of the known assay techniques useful for detecting mutations in a protein.

In vitro techniques for detection of the protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. Alternatively, the protein can be detected *in vivo* in a subject by introducing into the subject a labeled antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. Particularly useful are methods which detect the allelic variant of a protein of the invention expressed in a subject and methods which detect fragments of a protein of the invention in a sample.

- 10 The polypeptides of the invention are also useful in pharmacogenomic analysis. Pharmacogenomics deal with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, e.g., Eichelbaum, M., *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 (1996), and Linder, M.W., *Clin. Chem.* 43(2):254-266 (1997).
- 15 The clinical outcomes of these variations result in severe toxicity of therapeutic drugs in certain individuals or therapeutic failure of drugs in certain individuals as a result of individual variation in metabolism. Thus, the genotype of the individual can determine the way a therapeutic compound acts on the body or the way the body metabolizes the compound. Further, the activity of drug
- 20 metabolizing enzymes effects both the intensity and duration of drug action. Thus, the pharmacogenomics of the individual permit the selection of effective compounds and effective dosages of such compounds for prophylactic or therapeutic treatment based on the individual's genotype. The discovery of genetic polymorphisms in some drug metabolizing enzymes has explained why some
- 25 patients do not obtain the expected drug effects, show an exaggerated drug effect, or experience serious toxicity from standard drug dosages. Polymorphisms can be expressed in the phenotype of the extensive metabolizer and the phenotype of the poor metabolizer. Accordingly, genetic polymorphism may lead to allelic protein variants of the protein in which one or more of the protein functions in one
- 30 population is different from those in another population. The polypeptides thus allow a target to ascertain a genetic predisposition that can affect treatment modality. Thus, in a ligand-based treatment, polymorphism may give rise to

amino terminal extracellular domains and/or other ligand-binding regions that are more or less active in ligand binding, and receptor activation. Accordingly, ligand dosage would necessarily be modified to maximize the therapeutic effect within a given population containing a polymorphism. As an alternative to genotyping,
5 specific polymorphic polypeptides could be identified.

The polypeptides of the invention are also useful for monitoring therapeutic effects during clinical trials and other treatment. Thus, the therapeutic effectiveness of an agent that is designed to increase or decrease gene expression, protein levels or activity can be monitored over the course of treatment using the
10 polypeptides as an end-point target. The monitoring can be, for example, as follows: (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression or activity of a specified protein in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression
15 or activity of the protein in the post-administration samples; (v) comparing the level of expression or activity of the protein in the pre-administration sample with the protein in the post-administration sample or samples; and (vi) increasing or decreasing the administration of the agent to the subject accordingly.

The polypeptides of the invention are also useful for treating an associated
20 disorder. Accordingly, methods for treatment include the use of soluble protein or fragments of the protein that compete for ligand binding. These proteins or fragments can have a higher affinity for the ligand so as to provide effective competition.

25 Antibodies

The invention also provides antibodies that selectively bind to the 39404, 38911, and 26904 proteins and variants and fragments. An antibody is considered to selectively bind, even if it also binds to other proteins that are not substantially homologous with the proteins. These other proteins share homology with a
30 fragment or domain of the protein of the invention. This conservation in specific regions gives rise to antibodies that bind to both proteins by virtue of the

homologous sequence. In this case, it would be understood that antibody binding to the protein of the invention is still selective.

To generate antibodies, an isolated polypeptide of the invention is used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. Either the full-length protein or antigenic peptide fragment can be used. Regions having a high antigenicity index are shown in Figures 3, 9, and 15.

Antibodies are preferably prepared from these regions or from discrete fragments in these regions. However, antibodies can be prepared from any region of the peptide as described herein. A preferred fragment produces an antibody that diminishes or completely prevents ligand-binding. Antibodies can be developed against the entire protein or portions of the protein, for example, the intracellular carboxy terminal domain, the amino terminal extracellular domain, the entire transmembrane domain or specific segments, any of the intra or extracellular loops, or any portions of the above. Antibodies may also be developed against specific functional sites, such as the site of ligand-binding, the site of G protein coupling, or sites that are phosphorylated, glycosylated, or myristoylated.

An antigenic 39404, 38911, or 26904 fragment will typically comprise at least 8-10 contiguous amino acid residues. The antigenic peptide can comprise, however, a contiguous sequence of at least 12, 14 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, or at least 30 amino acid residues. In one embodiment, fragments correspond to regions that are located on the surface of the protein, e.g., hydrophilic regions. These fragments are not to be construed, however, as encompassing any fragments which may be disclosed prior to the invention.

Antibodies can be polyclonal or monoclonal. An intact antibody, or a fragment thereof (e.g. Fab or $F(ab)_2$) can be used.

Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or

acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example
5 of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

An appropriate immunogenic preparation can be derived from native, recombinantly expressed, protein or chemically synthesized peptides.

10

Antibody Uses

The antibodies can be used to isolate a protein of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. The antibodies can facilitate the purification of the natural protein from cells and
15 recombinantly produced protein expressed in host cells.

The antibodies are useful to detect the presence of a protein of the invention in cells or tissues to determine the pattern of expression of the protein among various tissues in an organism and over the course of normal development.

The antibodies can be used to detect a protein of the invention *in situ*, *in vitro*, or in a cell lysate or supernatant in order to evaluate the abundance and
20 pattern of expression.

The antibodies can be used to assess abnormal tissue distribution or abnormal expression during development.

Antibody detection of circulating fragments of the full length protein can
25 be used to identify protein turnover.

Further, the antibodies can be used to assess expression of a protein of the invention in disease states such as in active stages of the disease or in an individual with a predisposition toward disease related to protein function. When a disorder is caused by an inappropriate tissue distribution, developmental expression, or
30 level of expression of the protein, the antibody can be prepared against the normal protein. If a disorder is characterized by a specific mutation in the protein,

antibodies specific for this mutant protein can be used to assay for the presence of the specific mutant protein.

The antibodies can also be used to assess normal and aberrant subcellular localization of cells in the various tissues in an organism. Antibodies can be developed against the whole protein or portions of the receptor, for example, portions of the amino terminal extracellular domain or extracellular loops.

The diagnostic uses can be applied, not only in genetic testing, but also in monitoring a treatment modality. Accordingly, where treatment is ultimately aimed at correcting protein expression level or the presence of aberrant proteins of the invention and aberrant tissue distribution or developmental expression, antibodies directed against the protein or relevant fragments can be used to monitor therapeutic efficacy.

Antibodies accordingly can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen.

Additionally, antibodies are useful in pharmacogenomic analysis. Thus, antibodies prepared against polymorphic proteins of the invention can be used to identify individuals that require modified treatment modalities.

The antibodies are also useful as diagnostic tools as an immunological marker for aberrant protein analyzed by electrophoretic mobility, isoelectric point, tryptic peptide digest, and other physical assays known to those in the art.

The antibodies are also useful for tissue typing. Thus, where a specific protein has been correlated with expression in a specific tissue, antibodies that are specific for this protein can be used to identify a tissue type.

The antibodies are also useful in forensic identification. Accordingly, where an individual has been correlated with a specific genetic polymorphism resulting in a specific polymorphic protein, an antibody specific for the polymorphic protein can be used as an aid in identification.

The antibodies are also useful for inhibiting protein function, for example, blocking ligand binding.

These uses can also be applied in a therapeutic context in which treatment involves inhibiting protein function. An antibody can be used, for example, to

block ligand binding. Antibodies can be prepared against specific fragments containing sites required for function or against intact protein associated with a cell.

5 Completely human antibodies are particularly desirable for therapeutic treatment of human patients. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S.
10 Patent 5,661,016; and U.S. Patent 5,545,806.

The invention also encompasses kits for using antibodies to detect the presence of a protein of the invention in a biological sample. The kit can comprise antibodies such as a labeled or labelable antibody and a compound or agent for
15 detecting the protein in a biological sample; means for determining the amount of the protein in the sample; and means for comparing the amount of the protein in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the protein.

20 Polynucleotides

The nucleotide sequence in SEQ ID NO:2 was obtained by sequencing the deposited human full length cDNA. Accordingly, the sequence of the deposited clone is controlling as to any discrepancies between the two and any reference to the sequence of SEQ ID NO:2 includes reference to the sequence of the deposited
25 cDNA.

The nucleotide sequence in SEQ ID NO:4 was obtained by sequencing the deposited human full length cDNA. Accordingly, the sequence of the deposited clone is controlling as to any discrepancies between the two and any reference to the sequence of SEQ ID NO:4 includes reference to the sequence of the deposited
30 cDNA.

The nucleotide sequence in SEQ ID NO:6 was obtained by sequencing the deposited human full length cDNA. Accordingly, the sequence of the deposited

clone is controlling as to any discrepancies between the two and any reference to the sequence of SEQ ID NO:6 includes reference to the sequence of the deposited cDNA.

5 The specifically disclosed cDNAs comprise the coding region and 5' and 3' untranslated sequences (SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6).

The human 39404 cDNA is approximately 1729 nucleotides in length and encodes a full length protein that is approximately 337 amino acid residues in length. The nucleic acid is expressed in the tissues shown in Figures 5-7. Structural analysis of the amino acid sequence of SEQ ID NO:1 is provided in
10 Figure 2, a hydropathy plot. The figure shows the putative structure of the seven transmembrane segments, the amino terminal extracellular domain and the carboxy terminal intracellular domain.

The human 38911 cDNA is approximately 1334 nucleotides in length and encodes a full length protein that is approximately 337 amino acid residues in
15 length. The nucleic acid is expressed in the tissues shown in Figures 12 and 13. Structural analysis of the amino acid sequence of SEQ ID NO:3 is provided in Figure 9, a hydropathy plot. The figure shows the putative structure of the seven transmembrane segments, the amino terminal extracellular domain and the carboxy terminal intracellular domain.

20 The human 26904 cDNA is approximately 1743 nucleotides in length and encodes a full length protein that is approximately 450 amino acid residues in length. Structural analysis of the amino acid sequence of SEQ ID NO:5 is provided in Figure 14, a hydropathy plot. The figure shows the putative structure of the seven transmembrane segments, the amino terminal extracellular domain
25 and the carboxy terminal intracellular domain.

As used herein, the term "transmembrane segment" refers to a structural amino acid motif which includes a hydrophobic helix that spans the plasma membrane. The entire transmembrane domain of 39404 spans from about amino acid 38 to about amino acid 305. The entire transmembrane domain of 38911
30 spans from about amino acid 41 to about amino acid 294. The entire transmembrane domain of 26904 spans from about amino acid 30 to about amino acid 430.

The invention provides isolated polynucleotides encoding a 39404 protein. The term "39404 polynucleotide" or "39404 nucleic acid" refers to the sequence shown in SEQ ID NO:2 or in the deposited cDNA.

The invention provides isolated polynucleotides encoding a 38911 protein.
5 The term "38911 polynucleotide" or "38911 nucleic acid" refers to the sequence shown in SEQ ID NO:4 or in the deposited cDNA.

The invention provides isolated polynucleotides encoding a 26904 protein. The term "26904 polynucleotide" or "26904 nucleic acid" refers to the sequence shown in SEQ ID NO:6 or in the deposited cDNA.

10 The term "polynucleotide" or "nucleic acid" further includes variants and fragments of the 39404, 38911, and 26904 polynucleotides.

An "isolated" nucleic acid of the invention is one that is separated from other nucleic acid present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid
15 (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. However, there can be some flanking nucleotide sequences, for example up to about 5KB. The important point is that the nucleic acid is isolated from flanking sequences such that it can be subjected to the specific manipulations described herein such as
20 recombinant expression, preparation of probes and primers, and other uses specific to the nucleic acid sequences of the invention.

Moreover, an "isolated" nucleic acid molecule, such as a cDNA or RNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other
25 chemicals when chemically synthesized. However, the nucleic acid molecule can be fused to other coding or regulatory sequences and still be considered isolated.

For example, recombinant DNA molecules contained in a vector are considered isolated. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified
30 (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the isolated DNA molecules of the

present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system or reagent mix. In other circumstances, the material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC. Preferably, an isolated nucleic acid comprises at least about 50, 80 or 90 % (on a molar basis) of all macromolecular species present.

The polynucleotides of the invention can encode the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, facilitate protein trafficking, prolong or shorten protein half-life or facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

The polynucleotides of the invention include, but are not limited to, the sequence encoding the mature polypeptide alone, the sequence encoding the mature polypeptide and additional coding sequences, such as a leader or secretory sequence (e.g., a pre-pro or pro-protein sequence), the sequence encoding the mature polypeptide, with or without the additional coding sequences, plus additional non-coding sequences, for example introns and non-coding 5' and 3' sequences such as transcribed but non-translated sequences that play a role in transcription, mRNA processing (including splicing and polyadenylation signals), ribosome binding and stability of mRNA. In addition, the polynucleotide may be fused to a marker sequence encoding, for example, a peptide that facilitates purification.

Polynucleotides of the invention can be in the form of RNA, such as mRNA, or in the form DNA, including cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The nucleic acid, especially DNA, can be double-stranded or single-stranded.

Single-stranded nucleic acid can be the coding strand (sense strand) or the non-coding strand (anti-sense strand).

One nucleic acid comprises the nucleotide sequence shown in SEQ ID NO:2, corresponding to human 39404 cDNA.

5 One nucleic acid comprises the nucleotide sequence shown in SEQ ID NO:4, corresponding to human 38911 cDNA.

One nucleic acid comprises the nucleotide sequence shown in SEQ ID NO:6, corresponding to human 26904 cDNA.

In one embodiment, the nucleic acid comprises only the coding region.

10 The invention further provides variant polynucleotides, and fragments thereof, that differ from the nucleotide sequence shown in SEQ ID NO:2 due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequence shown in SEQ ID NO:2.

The invention further provides variant polynucleotides, and fragments thereof, that differ from the nucleotide sequence shown in SEQ ID NO:4 due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequence shown in SEQ ID NO:4.

The invention further provides variant polynucleotides, and fragments thereof, that differ from the nucleotide sequence shown in SEQ ID NO:6 due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequence shown in SEQ ID NO:6.

The invention also provides nucleic acid molecules encoding the variant polypeptides described herein. Such polynucleotides may be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism), or may be constructed by recombinant DNA methods or by chemical synthesis. Such non-naturally occurring variants may be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. Accordingly, as discussed above, the variants can contain nucleotide substitutions, deletions, inversions and insertions.

30 Typically, variants have a substantial identity with a nucleic acid molecule selected from the group consisting of SEQ ID NOS:2, 4, 6, and 8 and the complements thereof.

Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions.

Orthologs, homologs, and allelic variants can be identified using methods well known in the art. 39404 variants comprise a nucleotide sequence encoding a protein that is 40-45%, 45-50%, 50-55%, 55-60%, typically at least about 60-65%, 65-70%, or 70-75%, more typically at least about 70-75%, 75-80%, or 80-85%, and most typically at least about 85-90% or 90-95% or more homologous to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of the sequence.

38911 variants comprise a nucleotide sequence encoding a protein that is 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, typically at least about 70-75%, more typically at least about 75-80% or 80-85%, and most typically at least about 85-90% or 90-95% or more homologous to the nucleotide sequence shown in SEQ ID NO:4 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:4 or a fragment of the sequence.

26904 variants comprise a nucleotide sequence encoding a protein that is 50-55%, 55-60%, 60-65%, 65-70%, typically at least about 70-75%, more typically at least about 75-80% or 80-85%, and most typically at least about 85-90% or 90-95% or more homologous to the nucleotide sequence shown in SEQ ID NO:6 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:6 or a fragment of the sequence.

It is understood that stringent hybridization does not indicate substantial homology where it is due to general homology, such as poly A sequences, or sequences common to all or most proteins, all seven-transmembrane proteins, all GPCRs, or all family I GPCRs or even all purinoceptors or C5a receptors.

Moreover, it is understood that variants do not include any of the nucleic acid sequences that may have been disclosed prior to the invention.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which

5 nucleotide sequences encoding a polypeptide at least 50-55%, 55% homologous to each other typically remain hybridized to each other. The conditions can be such that sequences at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95% or more identical to each other remain hybridized to one another. Such stringent conditions are known to those

10 skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, incorporated by reference. One example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45EC, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65EC. In another non-limiting example, nucleic acid

15 molecules are allowed to hybridize in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more low stringency washes in 0.2X SSC/0.1% SDS at room temperature, or by one or more moderate stringency washes in 0.2X SSC/0.1% SDS at 42°C, or washed in 0.2X SSC/0.1% SDS at 65°C for high stringency. In one embodiment, an isolated nucleic acid molecule that hybridizes

20 under stringent conditions to the sequence of SEQ ID NOS:2, 4, 6, or 8 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). Timing of hybridization can vary from ½ hour to 10 hours or longer. Shorter

25 hybridizations however can include from 1 to 5, and 6 to 10 hours. Typically, hybridization is performed overnight for around 10-12 hours. The time of washes can also vary from around 10 minutes to 30 minutes. Typically, washes are performed from 10-20 minutes.

As understood by those of ordinary skill, the exact conditions can be

30 determined empirically and depend on ionic strength, temperature and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS. Other factors considered in determining the desired hybridization

conditions include the length of the nucleic acid sequences, base composition, percent mismatch between the hybridizing sequences and the frequency of occurrence of subsets of the sequences within other non-identical sequences.

Thus, equivalent conditions can be determined by varying one or more of these
5 parameters while maintaining a similar degree of identity or similarity between the two nucleic acid molecules.

The present invention also provides isolated nucleic acids that contain a single or double stranded fragment or portion that hybridizes under stringent conditions to a nucleotide sequence selected from the group consisting of SEQ ID
10 NOS:2, 4, 6, or 8 and the complements of SEQ ID NOS:2, 4, 6, or 8. In one embodiment, the nucleic acid consists of a portion of a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, or 8 and the complements SEQ ID NOS:2, 4, 6, or 8. The nucleic acid fragments of the invention are at least about 15, preferably at least about 18, 20, 23 or 25
15 nucleotides, and can be 30, 40, 50, 100, 200 or more nucleotides in length. Longer fragments, for example, 30 or more nucleotides in length, which encode antigenic proteins or polypeptides described herein are useful.

Furthermore, the invention provides polynucleotides that comprise a fragment of the full length polynucleotides of the invention. The fragment can be
20 single or double stranded and can comprise DNA or RNA. The fragment can be derived from either the coding or the non-coding sequence.

In one embodiment, an isolated 39404 nucleic acid is at least 23 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:2. The isolated
25 fragments can be at least between 5-10, 10-20, 20-30, 30-40, 40-50, etc. including but not limited to 50, 75, 100, 200, 250, or 500 nucleotides in length or greater.

In another embodiment, an isolated 38911 nucleic acid from around nucleotide 1 to around nucleotide 200 is at least 5 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the
30 nucleotide sequence of SEQ ID NO:4. In other embodiments, the isolated nucleic acid is from around nucleotide 950 to nucleotide 1080 and is at least five nucleotides in length, hybridizing under stringent conditions. In other

embodiments, from about nucleotide 190 to about nucleotide 950, fragments can be at least 5-10 nucleotides, at least 10-15 nucleotides, at least 15-20 nucleotides, at least 20-25 nucleotides, at least 25-30 nucleotides, at least 30-35 nucleotides, at least 35-40 nucleotides, for example, greater than 13 nucleotides, greater than 14
5 nucleotides, and greater than 18 nucleotides. In other embodiments, the nucleic acid is at least 40, 50, 100, 250, or 500 nucleotides in length or greater.

In another embodiment, an isolated 26904 nucleic acid from nucleotide 1 to around nucleotide 498 is at least 14 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide
10 sequence of SEQ ID NO:6. In another embodiment, the nucleic acid from around nucleotide 691 to around 1014 is at least 14 nucleotides. In other embodiments, the nucleic acid is at least 40, 50, 100, 250, or 500 nucleotides in length or greater.

In another embodiment, an isolated 39404 nucleic acid encodes the entire coding region from amino acid 1 to amino acid 337. In another embodiment, the
15 isolated 39404 nucleic acid encodes a sequence corresponding to the mature protein from about amino acid 6 to about amino acid 337. In another embodiment, an isolated 38911 nucleic acid encodes the entire coding region from amino acid 1 to amino acid 337. In another embodiment, the isolated 38911 nucleic acid encodes a sequence corresponding to the mature protein from about amino acid 6
20 to about amino acid 337. In another embodiment, an isolated 26904 nucleic acid encodes the entire coding region from amino acid 1 to amino acid 450. In another embodiment, the isolated 26904 nucleic acid encodes a sequence corresponding to the mature protein from about amino acid 6 to about amino acid 450.

Other fragments of all four proteins include nucleotide sequences encoding
25 the amino acid fragments described herein. Further, fragments can include subfragments of the specific domains or sites described herein. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments, according to the present invention, are not to be construed as encompassing those fragments that
30 may have been disclosed prior to the invention except as they are used in methods involving tissues/disorders with which gene expression is associated.

However, it is understood that a nucleic acid fragment includes any nucleic acid sequence that does not include the entire gene.

39404 nucleic acid fragments further include sequences corresponding to the domains described herein, subregions also described, and specific functional sites. 39404 nucleic acid fragments include but are not limited to nucleic acid molecules encoding a polypeptide comprising the amino terminal extracellular domain, comprising the region spanning the transmembrane domain, a polypeptide comprising the carboxy terminal intracellular domain, and a polypeptide encoding the G-protein receptor signature (130-132 or surrounding amino acid residues from about 120 to about 140), nucleic acid molecules encoding any of the seven transmembrane segments, extracellular or intracellular loops, glycosylation sites or phosphorylation sites.

38911 nucleic acid fragments include but are not limited to nucleic acid molecules encoding a polypeptide comprising the amino terminal extracellular domain, the region spanning the transmembrane domain, and/or the carboxy terminal intracellular domain, and nucleic acid molecules encoding any of the seven transmembrane segments, extracellular or intracellular loops, glycosylation sites and phosphorylation sites.

26904 nucleic acid fragments include but are not limited to nucleic acid molecules encoding a polypeptide comprising the amino terminal extracellular domain, a polypeptide comprising the region spanning the transmembrane domain, and/or the carboxy terminal intracellular domain, and nucleic acid molecules encoding any of the seven transmembrane segments, extracellular or intracellular loops, glycosylation sites, protein kinase C, cAMP, cGMP, and casein kinase II phosphorylation sites, and myristoylation sites.

Where the location of the domains have been predicted by computer analysis, one of ordinary skill would appreciate that the amino acid residues constituting these domains can vary depending on the criteria used to define the domains.

Nucleic acid fragments also include combinations of the domains, segments, loops, and other functional sites described above. Thus, for example, a nucleic acid could include sequences corresponding to the amino terminal

extracellular domain and one transmembrane segment. A person of ordinary skill in the art would be aware of the many permutations that are possible.

Where the location of the domains or sites have been predicted by computer analysis, one of ordinary skill would appreciate that the amino acid
5 residues constituting these domains can vary depending on the criteria used to define the domains.

The invention also provides nucleic acid fragments that encode epitope bearing regions of the proteins described herein.

The isolated polynucleotide sequences, and especially fragments, are
10 useful as DNA probes and primers.

For example, the coding region of a gene of the invention can be isolated using the known nucleotide sequence to synthesize an oligonucleotide probe. A labeled probe can then be used to screen a cDNA library, genomic DNA library, or mRNA to isolate nucleic acid corresponding to the coding region. Further,
15 primers can be used in PCR reactions to clone specific regions of the genes of the invention.

A probe/primer typically comprises substantially purified oligonucleotide. The 39404 oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 10, 20, typically about
20 25, more typically about 40, 50 or 75 consecutive nucleotides of SEQ ID NO:2 sense or anti-sense strand or other receptor polynucleotides. The 38911 oligonucleotide typically comprises a region of nucleotide sequence from 1-1080 that hybridizes under stringent conditions to at least about 15, typically about 25, more typically about 40, 50 or 75 consecutive nucleotides of SEQ ID NO:4 sense
25 or anti-sense strand or other polynucleotides. The 26904 oligonucleotide typically comprises a region of nucleotide sequence from 1-498 of at least about 14, typically about 25, more typically about 40, 50 or 75 consecutive nucleotides of SEQ ID NO:6 sense or anti-sense strand or other polynucleotides that hybridizes under stringent conditions. The 26904 oligonucleotide also typically comprises a
30 region of nucleotide sequence from nucleotide 691-1014 at least about 14, typically about 25, more typically about 40, 50, or 75 consecutive nucleotides of

SEQ ID NO:6 sense or anti-sense strand or other polynucleotides that hybridizes under stringent conditions.

Polynucleotide Uses

5 The nucleic acid sequences of the present invention can be used as a “query sequence” to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be
10 performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default
15 parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

 The nucleic acid fragments of the invention provide probes or primers in assays such as those described below. “Probes” are oligonucleotides that hybridize in a base-specific manner to a complementary strand of nucleic acid. Such probes
20 include polypeptide nucleic acids, as described in Nielsen *et al.* (1991) *Science* 254:1497-1500. Typically, a probe comprises a region of nucleotide sequence that hybridizes under highly stringent conditions to at least about 15, typically about 20-25, and more typically about 40, 50 or 75 consecutive nucleotides of a nucleic acid selected from the group consisting of SEQ ID NOS:2, 4, 6 or 8 and the
25 complements thereof. More typically, the probe further comprises a label, e.g., radioisotope, fluorescent compound, enzyme, or enzyme co-factor.

 As used herein, the term “primer” refers to a single-stranded oligonucleotide which acts as a point of initiation of template-directed DNA synthesis using well-known methods (e.g., PCR, LCR) including, but not limited
30 to those described herein. The appropriate length of the primer depends on the particular use, but typically ranges from about 15 to 30 nucleotides. The term “primer site” refers to the area of the target DNA to which a primer hybridizes.

The term "primer pair" refers to a set of primers including a 5' (upstream) primer that hybridizes with the 5' end of the nucleic acid sequence to be amplified and a 3' (downstream) primer that hybridizes with the complement of the sequence to be amplified.

5 The polynucleotides are useful for various biological assays as described in detail below. Since the 39404 gene is expressed in the tissues shown in Figures 5-7, the assays are particularly useful in cells derived from these tissue types, and particularly the tissues in which the gene is highly expressed, such as brain, kidney, fetal kidney, fetal liver, internal mammary artery, and aortic intimal
10 proliferations. Furthermore, since the gene is expressed in these tissues, assays involving the polynucleotides in pathological tissue/disorders, particularly applies to disorders involving these tissues and especially the tissues in which the gene is highly expressed. Moreover, since the gene is expressed in aortic intimal
15 proliferations (atheroplaques), and heart tissue from patients with congestive heart failure, ischemia, and myopathy, the assays and methods involving pathology/disorders are particularly relevant in these disorders.

 Since the 38911 is expressed in the tissues shown in Figures 12 and 13, the assays are particularly useful in cells derived from these tissue types, and particularly the tissues in which the gene is highly expressed, such as kidney,
20 spleen, fibrotic liver tissue, tonsils, osteoclasts, liver, and testis. Furthermore, since the gene is expressed in these tissues, assays involving the polynucleotides and pathological tissues/disorders, particularly applies to disorders involving these tissues and especially the tissues in which the gene is highly expressed. Moreover, since the gene is expressed in liver fibrosis, the assays and methods involving
25 pathology/disorders are particularly relevant in these disorders. Finally, in view of the fact that the gene is highly expressed in osteoclasts, assays and methods involving osteoporosis are particularly relevant.

 The receptor polynucleotides are useful for probes, primers, and in biological assays.

30 Where the polynucleotides are used to assess seven-transmembrane protein/GPCR properties or functions, such as in the assays described herein, all or less than all of the entire cDNA can be useful. In this case, even fragments that

may have been known prior to the invention are encompassed. Thus, for example, assays specifically directed to seven-transmembrane protein/GPCR functions, such as assessing agonist or antagonist activity, encompass the use of known fragments. Further, diagnostic methods for assessing protein function can also be practiced
5 with any fragment, including those fragments that may have been known prior to the invention. Similarly, in methods involving treatment of protein dysfunction, all fragments are encompassed including those which may have been known in the art.

The 39404 polynucleotides are useful as a hybridization probe for cDNA
10 and genomic DNA to isolate a full-length cDNA and genomic clones encoding the polypeptide described in SEQ ID NO:1 and to isolate cDNA and genomic clones that correspond to variants producing the same polypeptide shown in SEQ ID NO:1 or the other variants described herein. Variants can be isolated from the same tissue and organism from which the polypeptide shown in SEQ ID NO:1
15 was isolated, different tissues from the same organism, or from different organisms.

The 38911 polynucleotides are useful as a hybridization probe for cDNA and genomic DNA to isolate a full-length cDNA and genomic clones encoding the polypeptide described in SEQ ID NO:3 and to isolate cDNA and genomic clones
20 that correspond to variants producing the same polypeptide shown in SEQ ID NO:3 or the other variants described herein. Variants can be isolated from the same tissue and organism from which the polypeptide shown in SEQ ID NO:3 was isolated, different tissues from the same organism, or from different organisms.

25 The 26904 polynucleotides are useful as a hybridization probe for cDNA and genomic DNA to isolate a full-length cDNA and genomic clones encoding the polypeptide described in SEQ ID NO:5 and to isolate cDNA and genomic clones that correspond to variants producing the same polypeptide shown in SEQ ID NO:5 or the other variants described herein. Variants can be isolated from the
30 same tissue and organism from which the polypeptide shown in SEQ ID NO:5 was isolated, different tissues from the same organism, or from different organisms.

This method is useful for isolating genes and cDNA that are developmentally-controlled and therefore may be expressed in the same tissue or different tissues at different points in the development of an organism.

5 The probe can correspond to any sequence along the entire length of the gene encoding the protein. Accordingly, it could be derived from 5' noncoding regions, the coding region, and 3' noncoding regions. Probes, however, are not to be construed as corresponding to any sequences that may be known prior to the invention.

10 The 39404 nucleic acid probe can be, for example, the full-length cDNA of SEQ ID NO:1, or a fragment thereof, such as an oligonucleotide of at least 10, 20, 30, 40, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to mRNA or DNA. The 38911 nucleic acid probe can be, for example, the full-length cDNA of SEQ ID NO:3, or a fragment thereof, such as an oligonucleotide of at least 10, 20, 30, 40, 50, 100, 250 or 500
15 nucleotides in length and sufficient to specifically hybridize under stringent conditions to mRNA or DNA. The 26904 nucleic acid probe can be, for example, the full-length cDNA of SEQ ID NO:5, or a fragment thereof, such as an oligonucleotide of at least 10, 20, 30, 40, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to mRNA or
20 DNA.

Fragments of the polynucleotides described herein are also useful to synthesize larger fragments or full-length polynucleotides described herein. For example, a fragment can be hybridized to any portion of an mRNA and a larger or full-length cDNA can be produced.

25 The fragments are also useful to synthesize antisense molecules of desired length and sequence.

Antisense nucleic acids of the invention can be designed using the nucleotide sequences of SEQ ID NOS:2, 4, 6, or 8, and constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For
30 example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules

or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest.

Additionally, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4:5). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996), *supra*; Perry-O'Keefe *et al.*

(1996) *Proc. Natl. Acad. Sci. USA* 93:14670. PNAs can be further modified, e.g., to enhance their stability, specificity or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. The
5 synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), *supra*, Finn *et al.* (1996) *Nucleic Acids Res.* 24(17):3357-63, Mag *et al.* (1989) *Nucleic Acids Res.* 17:5973, and Peterser *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5:1119.

The nucleic acid molecules and fragments of the invention can also include
10 other appended groups such as peptides (e.g., for targeting host cell proteins *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO 88/0918) or the blood brain barrier (see, e.g., PCT Publication No. WO 89/10134). In addition,
15 oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents (see, e.g., Zon (1988) *Pharm Res.* 5:539-549).

The polynucleotides of the invention are also useful as primers for PCR to amplify any given region of the polynucleotide.

20 The polynucleotides are also useful for constructing recombinant vectors. Such vectors include expression vectors that express a portion of, or all of, the polypeptides. Vectors also include insertion vectors, used to integrate into another polynucleotide sequence, such as into the cellular genome, to alter *in situ* expression of the genes and gene products. For example, an endogenous coding
25 sequence can be replaced via homologous recombination with all or part of the coding region containing one or more specifically introduced mutations.

The polynucleotides are also useful for expressing antigenic peptides. Peptide regions having a high antigenicity index are shown in Figures 3, 9, and 15.

The polynucleotides are also useful as probes for determining the
30 chromosomal positions of the polynucleotides of the invention by means of *in situ* hybridization methods, such as FISH (for a review of this technique, see Verma *et al.* (1988) *Human Chromosomes: A Manual of Basic Techniques* (Pergamon

Press, New York), and PCR mapping of somatic cell hybrids. The mapping of the sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland *et al.* (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a specified gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The polynucleotide probes are also useful to determine patterns of the presence of the gene encoding the proteins of the invention and their variants with respect to tissue distribution, for example, whether gene duplication has occurred and whether the duplication occurs in all or only a subset of tissues. The genes

can be naturally occurring or can have been introduced into a cell, tissue, or organism exogenously.

The polynucleotides are also useful for designing ribozymes corresponding to all, or a part, of the mRNA produced from genes encoding the polynucleotides described herein.

The polynucleotides are also useful for constructing host cells expressing a part, or all, of the polynucleotides and polypeptides of the invention.

The polynucleotides are also useful for constructing transgenic animals expressing all, or a part, of the polynucleotides and polypeptides of the invention.

The polynucleotides are also useful for making vectors that express part, or all, of the polypeptides of the invention.

The polynucleotides are also useful as hybridization probes for determining the level of nucleic acid expression of the nucleic acid molecules of the invention. Accordingly, the probes can be used to detect the presence of, or to determine levels of, the nucleic acid in cells, tissues, and in organisms. The nucleic acid whose level is determined can be DNA or RNA. Accordingly, probes corresponding to the polypeptides described herein can be used to assess gene copy number in a given cell, tissue, or organism. This is particularly relevant in cases in which there has been an amplification of the genes of the invention.

Alternatively, the probe can be used in an *in situ* hybridization context to assess the position of extra copies of the genes of the invention, as on extrachromosomal elements or as integrated into chromosomes in which the gene is not normally found, for example as a homogeneously staining region.

These uses are relevant for diagnosis of disorders involving an increase or decrease in expression relative to normal, such as a proliferative disorder, a differentiative or developmental disorder, or a hematopoietic disorder.

Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant expression or activity of a nucleic acid of the invention, in which a test sample is obtained from a subject and nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of the nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant expression or activity of the nucleic acid.

One aspect of the invention relates to diagnostic assays for determining nucleic acid expression as well as activity in the context of a biological sample (e.g., blood, serum, cells, tissue) to determine whether an individual has a disease or disorder, or is at risk of developing a disease or disorder, associated with aberrant nucleic acid expression or activity. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with expression or activity of the nucleic acid molecules.

In vitro techniques for detection of mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detecting DNA includes Southern hybridizations and *in situ* hybridization.

Probes can be used as a part of a diagnostic test kit for identifying cells or tissues that express a protein of the invention, such as by measuring a level of a protein-encoding nucleic acid in a sample of cells from a subject e.g., mRNA or genomic DNA, or determining if a gene of the invention has been mutated.

Nucleic acid expression assays are useful for drug screening to identify compounds that modulate nucleic acid expression (e.g., antisense, polypeptides, peptidomimetics, small molecules or other drugs) of the nucleic acid molecules of the invention. A cell is contacted with a candidate compound and the expression of mRNA determined. The level of expression of mRNA of the invention in the presence of the candidate compound is compared to the level of expression of the mRNA in the absence of the candidate compound. The candidate compound can then be identified as a modulator of nucleic acid expression based on this comparison and be used, for example to treat a disorder characterized by aberrant nucleic acid expression. The modulator can bind to the nucleic acid or indirectly modulate expression, such as by interacting with other cellular components that affect nucleic acid expression.

Modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject) in patients or in transgenic animals.

The invention thus provides a method for identifying a compound that can be used to treat a disorder associated with nucleic acid expression of the receptor

gene. The method typically includes assaying the ability of the compound to modulate the expression of the nucleic acid and thus identifying a compound that can be used to treat a disorder characterized by undesired nucleic acid expression of the nucleic acid molecules of the invention.

5 The assays can be performed in cell-based and cell-free systems. Cell-based assays include cells naturally expressing the nucleic acid or recombinant cells genetically engineered to express specific nucleic acid sequences.

Alternatively, candidate compounds can be assayed *in vivo* in patients or in transgenic animals.

10 The assay for nucleic acid expression can involve direct assay of nucleic acid levels, such as mRNA levels, or on collateral compounds involved in the signal pathway (such as cyclic AMP or phosphatidylinositol turnover). Further, the expression of genes that are up- or down-regulated in response to the protein signal pathway can also be assayed. In this embodiment the regulatory regions of
15 these genes can be operably linked to a reporter gene such as luciferase.

Thus, modulators of gene expression can be identified in a method wherein a cell is contacted with a candidate compound and the expression of mRNA determined. The level of expression of mRNA in the presence of the candidate compound is compared to the level of expression of mRNA in the absence of the
20 candidate compound. The candidate compound can then be identified as a modulator of nucleic acid expression based on this comparison and be used, for example to treat a disorder characterized by aberrant nucleic acid expression. When expression of mRNA is statistically significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified
25 as a stimulator of nucleic acid expression. When nucleic acid expression is statistically significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of nucleic acid expression.

Accordingly, the invention provides methods of treatment, with the nucleic
30 acid as a target, using a compound identified through drug screening as a gene modulator to modulate nucleic acid expression of the nucleic acid molecules of the invention. Modulation includes both up-regulation (i.e. activation or agonization)

or down-regulation (suppression or antagonization) or effects on nucleic acid activity (e.g. when nucleic acid is mutated or improperly modified). Treatment is of disorders characterized by aberrant expression or activity of the nucleic acid.

Alternatively, a modulator of nucleic acid expression can be a small molecule or drug identified using the screening assays described herein as long as the drug or small molecule inhibits the nucleic acid expression.

The polynucleotides are also useful for monitoring the effectiveness of modulating compounds on the expression or activity of the gene in clinical trials or in a treatment regimen. Thus, the gene expression pattern can serve as a barometer for the continuing effectiveness of treatment with the compound, particularly with compounds to which a patient can develop resistance. The gene expression pattern can also serve as a marker indicative of a physiological response of the affected cells to the compound. Accordingly, such monitoring would allow either increased administration of the compound or the administration of alternative compounds to which the patient has not become resistant. Similarly, if the level of nucleic acid expression falls below a desirable level, administration of the compound could be commensurately decreased.

Monitoring can be, for example, as follows: (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a specified mRNA or genomic DNA of the invention in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the mRNA or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the mRNA or genomic DNA in the pre-administration sample with the mRNA or genomic DNA in the post-administration sample or samples; and (vi) increasing or decreasing the administration of the agent to the subject accordingly.

The polynucleotides of the invention are also useful in diagnostic assays for qualitative changes in the nucleic acid, and particularly in qualitative changes that lead to pathology. The polynucleotides can be used to detect mutations in genes of the invention and gene expression products such as mRNA. The polynucleotides can be used as hybridization probes to detect naturally-occurring

genetic mutations in the gene and thereby to determine whether a subject with the mutation is at risk for a disorder caused by the mutation. Mutations include deletion, addition, or substitution of one or more nucleotides in the gene, chromosomal rearrangement, such as inversion or transposition, modification of
5 genomic DNA, such as aberrant methylation patterns or changes in gene copy number, such as amplification. Detection of a mutated form of the gene associated with a dysfunction provides a diagnostic tool for an active disease or susceptibility to disease when the disease results from overexpression, underexpression, or altered expression of a protein of the invention.

10 Mutations in a gene of the invention can be detected at the nucleic acid level by a variety of techniques. Genomic DNA can be analyzed directly or can be amplified by using PCR prior to analysis. RNA or cDNA can be used in the same way.

In certain embodiments, detection of the mutation involves the use of a
15 probe/primer in a polymerase chain reaction (PCR) (see, e.g. U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.*, *Science* 241:1077-1080 (1988); and Nakazawa *et al.*, *PNAS* 91:360-364 (1994)), the latter of which can be particularly useful for detecting point mutations in the gene (see Abravaya *et al.*,
20 *Nucleic Acids Res.* 23:675-682 (1995)). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a gene under conditions such that hybridization and amplification of the gene (if present) occurs, and
25 detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. Deletions and insertions can be detected by a change in size of the amplified product compared to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to normal RNA or antisense DNA sequences.

30 It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well-known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

Alternatively, mutations in a gene of the invention can be directly identified, for example, by alterations in restriction enzyme digestion patterns determined by gel electrophoresis.

Further, sequence-specific ribozymes (U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

Perfectly matched sequences can be distinguished from mismatched sequences by nuclease cleavage digestion assays or by differences in melting temperature.

Sequence changes at specific locations can also be assessed by nuclease protection assays such as RNase and S1 protection or the chemical cleavage method.

Furthermore, sequence differences between a mutant gene of the invention and a wild-type gene can be determined by direct DNA sequencing. A variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.*, *Adv. Chromatogr.* 36:127-162 (1996); and Griffin *et al.*, *Appl. Biochem. Biotechnol.* 38:147-159 (1993)).

Other methods for detecting mutations in the gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA duplexes (Myers *et al.*, *Science* 230:1242 (1985)); Cotton *et al.*, *PNAS* 85:4397 (1988); Saleeba *et al.*, *Meth. Enzymol.* 217:286-295 (1992)), electrophoretic mobility of mutant and wild type nucleic acid is compared

(Orita *et al.*, *PNAS* 86:2766 (1989); Cotton *et al.*, *Mutat. Res.* 285:125-144 (1993); and Hayashi *et al.*, *Genet. Anal. Tech. Appl.* 9:73-79 (1992)), and movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (Myers *et al.*,
5 *Nature* 313:495 (1985)). The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet.* 7:5).
10 Examples of other techniques for detecting point mutations include, selective oligonucleotide hybridization, selective amplification, and selective primer extension.

In other embodiments, genetic mutations can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays
15 containing hundreds or thousands of oligonucleotide probes (Cronin *et al.* (1996) *Human Mutation* 7:244-255; Kozal *et al.* (1996) *Nature Medicine* 2:753-759). For example, genetic mutations can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA
20 in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation
25 array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

The polynucleotides of the invention are also useful for testing an individual for a genotype that while not necessarily causing the disease, nevertheless affects the treatment modality. Thus, the polynucleotides can be used
30 to study the relationship between an individual's genotype and the individual's response to a compound used for treatment (pharmacogenomic relationship). In the present case, for example, a mutation in the gene that results in altered affinity

for ligand could result in an excessive or decreased drug effect with standard concentrations of ligand that activates the protein. Accordingly, the polynucleotides described herein can be used to assess the mutation content of the gene in an individual in order to select an appropriate compound or dosage regimen for treatment.

Thus polynucleotides displaying genetic variations that affect treatment provide a diagnostic target that can be used to tailor treatment in an individual. Accordingly, the production of recombinant cells and animals containing these polymorphisms allow effective clinical design of treatment compounds and dosage regimens.

The methods can involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting mRNA, or genomic DNA, such that the presence of mRNA or genomic DNA is detected in the biological sample, and comparing the presence of mRNA or genomic DNA in the control sample with the presence of mRNA or genomic DNA in the test sample.

The polynucleotides are also useful for chromosome identification when the sequence is identified with an individual chromosome and to a particular location on the chromosome. First, the DNA sequence is matched to the chromosome by *in situ* or other chromosome-specific hybridization. Sequences can also be correlated to specific chromosomes by preparing PCR primers that can be used for PCR screening of somatic cell hybrids containing individual chromosomes from the desired species. Only hybrids containing the chromosome containing the gene homologous to the primer will yield an amplified fragment. Sublocalization can be achieved using chromosomal fragments. Other strategies include prescreening with labeled flow-sorted chromosomes and preselection by hybridization to chromosome-specific libraries. Further mapping strategies include fluorescence *in situ* hybridization which allows hybridization with probes shorter than those traditionally used. Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on the chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the

genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

5 The polynucleotides can also be used to identify individuals from small biological samples. This can be done for example using restriction fragment-length polymorphism (RFLP) to identify an individual. Thus, the polynucleotides described herein are useful as DNA markers for RFLP (See U.S. Patent No. 5,272,057).

10 Furthermore, the gene sequence can be used to provide an alternative technique which determines the actual DNA sequence of selected fragments in the genome of an individual. Thus, the receptor sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify DNA from an individual for subsequent sequencing.

15 Panels of corresponding DNA sequences from individuals prepared in this manner can provide unique individual identifications, as each individual will have a unique set of such DNA sequences. It is estimated that allelic variation in humans occurs with a frequency of about once per each 500 bases. Allelic variation occurs to some degree in the coding regions of these sequences, and to a
20 greater degree in the noncoding regions. The sequences can be used to obtain such identification sequences from individuals and from tissue. The sequences represent unique fragments of the human genome. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes.

25 If a panel of reagents from the sequences is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

30 The polynucleotides can also be used in forensic identification procedures. PCR technology can be used to amplify DNA sequences taken from very small biological samples, such as a single hair follicle, body fluids (e.g. blood, saliva, or

semen). The amplified sequence can then be compared to a standard allowing identification of the origin of the sample.

The polynucleotides can thus be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As described above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to the noncoding region are particularly useful since greater polymorphism occurs in the noncoding regions, making it easier to differentiate individuals using this technique.

The polynucleotides can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This is useful in cases in which a forensic pathologist is presented with a tissue of unknown origin. Panels of probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these primers and probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

Alternatively, the polynucleotides can be used directly to block transcription or translation of the gene sequences by means of antisense or ribozyme constructs. Thus, in a disorder characterized by abnormally high or undesirable expression of the gene of the invention, nucleic acids can be directly used for treatment.

The polynucleotides are thus useful as antisense constructs to control expression of a gene of the invention in cells, tissues, and organisms. A DNA antisense polynucleotide is designed to be complementary to a region of the gene involved in transcription, preventing transcription and hence production of the protein of the invention. An antisense RNA or DNA polynucleotide would hybridize to the mRNA and thus block translation of mRNA into protein.

Examples of antisense molecules useful to inhibit nucleic acid expression include antisense molecules complementary to a fragment of the 5' untranslated region of SEQ ID NOS:2, 4, or 6, which also includes the start codon and antisense molecules which are complementary to a fragment of the 3' untranslated region of SEQ ID NOS:2, 4, or 6.

Alternatively, a class of antisense molecules can be used to inactivate mRNA in order to decrease expression of nucleic acid of the invention. Accordingly, these molecules can treat a disorder characterized by abnormal or undesired expression of a nucleic acid of the invention. This technique involves cleavage by means of ribozymes containing nucleotide sequences complementary to one or more regions in the mRNA that attenuate the ability of the mRNA to be translated. Possible regions include coding regions and particularly coding regions corresponding to the catalytic and other functional activities of the protein of the invention, such as ligand binding.

The polynucleotides also provide vectors for gene therapy in patients containing cells that are aberrant in expression of a gene of the invention. Thus, recombinant cells, which include the patient's cells that have been engineered *ex vivo* and returned to the patient, are introduced into an individual where the cells produce the desired protein to treat the individual.

The invention also encompasses kits for detecting the presence of a nucleic acid of the invention in a biological sample. For example, the kit can comprise reagents such as a labeled or labelable nucleic acid or agent capable of detecting the nucleic acid in a biological sample; means for determining the amount of the nucleic acid in the sample; and means for comparing the amount of the nucleic acid in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the mRNA or DNA.

Computer Readable Means

The nucleotide or amino acid sequences of the invention are also provided in a variety of mediums to facilitate use thereof. As used herein, "provided" refers to a manufacture, other than an isolated nucleic acid or amino acid molecule,

which contains a nucleotide or amino acid sequence of the present invention. Such a manufacture provides the nucleotide or amino acid sequences, or a subset thereof (e.g., a subset of open reading frames (ORFs)) in a form which allows a skilled artisan to examine the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form.

In one application of this embodiment, a nucleotide or amino acid sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. The skilled artisan will readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. The skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the nucleotide or amino acid sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The

skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g., text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention.

Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBIA).

For example, software which implements the BLAST (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410) and BLAZE (Brutlag *et al.* (1993) *Comp. Chem.* 17:203-207) search algorithms on a Sybase system can be used to identify open reading frames (ORFs) of the sequences of the invention which contain homology to ORFs or proteins from other libraries. Such ORFs are protein encoding fragments and are useful in producing commercially important proteins such as enzymes used in various reactions and in the production of commercially useful metabolites.

Vectors/host cells

The invention also provides vectors containing the polynucleotides of the invention. The term "vector" refers to a vehicle, preferably a nucleic acid molecule, that can transport the polynucleotides. When the vector is a nucleic acid molecule, the polynucleotides are covalently linked to the vector nucleic acid. With this aspect of the invention, the vector includes a plasmid, single or double stranded phage, a single or double stranded RNA or DNA viral vector, or artificial chromosome, such as a BAC, PAC, YAC, OR MAC.

A vector can be maintained in the host cell as an extrachromosomal element where it replicates and produces additional copies of the polynucleotides of the invention. Alternatively, the vector may integrate into the host cell genome and produce additional copies of the polynucleotides when the host cell replicates.

The invention provides vectors for the maintenance (cloning vectors) or vectors for expression (expression vectors) of the polynucleotides. The vectors can function in procaryotic or eukaryotic cells or in both (shuttle vectors).

Expression vectors contain cis-acting regulatory regions that are operably linked in the vector to the polynucleotides such that transcription of the polynucleotides is allowed in a host cell. The polynucleotides can be introduced into the host cell with a separate polynucleotide capable of affecting transcription. Thus, the second polynucleotide may provide a trans-acting factor interacting with the cis-regulatory control region to allow transcription of the polynucleotides from

the vector. Alternatively, a trans-acting factor may be supplied by the host cell. Finally, a trans-acting factor can be produced from the vector itself.

It is understood, however, that in some embodiments, transcription and/or translation of the polynucleotides can occur in a cell-free system.

5 The regulatory sequence to which the polynucleotides described herein can be operably linked include promoters for directing mRNA transcription. These include, but are not limited to, the left promoter from bacteriophage λ , the lac, TRP, and TAC promoters from *E. coli*, the early and late promoters from SV40, the CMV immediate early promoter, the adenovirus early and late promoters, and
10 retrovirus long-terminal repeats.

 In addition to control regions that promote transcription, expression vectors may also include regions that modulate transcription, such as repressor binding sites and enhancers. Examples include the SV40 enhancer, the cytomegalovirus immediate early enhancer, polyoma enhancer, adenovirus
15 enhancers, and retrovirus LTR enhancers.

 In addition to containing sites for transcription initiation and control, expression vectors can also contain sequences necessary for transcription termination and, in the transcribed region a ribosome binding site for translation. Other regulatory control elements for expression include initiation and termination
20 codons as well as polyadenylation signals. The person of ordinary skill in the art would be aware of the numerous regulatory sequences that are useful in expression vectors. Such regulatory sequences are described, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989).

25 A variety of expression vectors can be used to express a polynucleotide of the invention. Such vectors include chromosomal, episomal, and virus-derived vectors, for example vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, including yeast artificial chromosomes, from viruses such as baculoviruses, papovaviruses such as SV40,
30 Vaccinia viruses, adenoviruses, poxviruses, pseudorabies viruses, and retroviruses. Vectors may also be derived from combinations of these sources such as those derived from plasmid and bacteriophage genetic elements, e.g. cosmids and

phagemids. Appropriate cloning and expression vectors for prokaryotic and eukaryotic hosts are described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989).

5 The regulatory sequence may provide constitutive expression in one or more host cells (i.e. tissue specific) or may provide for inducible expression in one or more cell types such as by temperature, nutrient additive, or exogenous factor such as a hormone or other ligand. A variety of vectors providing for constitutive and inducible expression in prokaryotic and eukaryotic hosts are well known to
10 those of ordinary skill in the art.

 The polynucleotides can be inserted into the vector nucleic acid by well-known methodology. Generally, the DNA sequence that will ultimately be expressed is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction enzymes and then ligating the
15 fragments together. Procedures for restriction enzyme digestion and ligation are well known to those of ordinary skill in the art.

 The vector containing the appropriate polynucleotide can be introduced into an appropriate host cell for propagation or expression using well-known techniques. Bacterial cells include, but are not limited to, *E. coli*, *Streptomyces*,
20 and *Salmonella typhimurium*. Eukaryotic cells include, but are not limited to, yeast, insect cells such as *Drosophila*, animal cells such as COS and CHO cells, and plant cells.

 As described herein, it may be desirable to express the polypeptide as a fusion protein. Accordingly, the invention provides fusion vectors that allow for
25 the production of the polypeptides. Fusion vectors can increase the expression of a recombinant protein, increase the solubility of the recombinant protein, and aid in the purification of the protein by acting for example as a ligand for affinity purification. A proteolytic cleavage site may be introduced at the junction of the fusion moiety so that the desired polypeptide can ultimately be separated from the
30 fusion moiety. Proteolytic enzymes include, but are not limited to, factor Xa, thrombin, and enterokinase. Typical fusion expression vectors include pGEX (Smith *et al.*, *Gene* 67:31-40 (1988)), pMAL (New England Biolabs, Beverly,

MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, *Gene* 69:301-315 (1988)) and pET 11d
5 (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185:60-89 (1990)).

Recombinant protein expression can be maximized in a host bacteria by providing a genetic background wherein the host cell has an impaired capacity to proteolytically cleave the recombinant protein. (Gottesman, S., *Gene Expression*
10 *Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Alternatively, the sequence of the polynucleotide of interest can be altered to provide preferential codon usage for a specific host cell, for example *E. coli*. (Wada *et al.*, *Nucleic Acids Res.* 20:2111-2118 (1992)).

The polynucleotides can also be expressed by expression vectors that are
15 operative in yeast. Examples of vectors for expression in yeast e.g., *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, *EMBO J.* 6:229-234 (1987)), pMFa (Kurjan *et al.*, *Cell* 30:933-943 (1982)), pJRY88 (Schultz *et al.*, *Gene* 54:113-123 (1987)), and pYES2 (Invitrogen Corporation, San Diego, CA).

The polynucleotides can also be expressed in insect cells using, for
20 example, baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith *et al.*, *Mol. Cell Biol.* 3:2156-2165 (1983)) and the pVL series (Lucklow *et al.*, *Virology* 170:31-39 (1989)).

In certain embodiments of the invention, the polynucleotides described
25 herein are expressed in mammalian cells using mammalian expression vectors. Examples of mammalian expression vectors include pCDM8 (Seed, B. *Nature* 329:840 (1987)) and pMT2PC (Kaufman *et al.*, *EMBO J.* 6:187-195 (1987)).

The expression vectors listed herein are provided by way of example only of the well-known vectors available to those of ordinary skill in the art that would
30 be useful to express the polynucleotides. The person of ordinary skill in the art would be aware of other vectors suitable for maintenance propagation or expression of the polynucleotides described herein. These are found for example

in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual, 2nd, ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

The invention also encompasses vectors in which the nucleic acid
5 sequences described herein are cloned into the vector in reverse orientation, but operably linked to a regulatory sequence that permits transcription of antisense RNA. Thus, an antisense transcript can be produced to all, or to a portion, of the polynucleotide sequences described herein, including both coding and non-coding regions. Expression of this antisense RNA is subject to each of the parameters
10 described above in relation to expression of the sense RNA (regulatory sequences, constitutive or inducible expression, tissue-specific expression).

The invention also relates to recombinant host cells containing the vectors described herein. Host cells therefore include prokaryotic cells, lower eukaryotic cells such as yeast, other eukaryotic cells such as insect cells, and higher
15 eukaryotic cells such as mammalian cells.

The recombinant host cells are prepared by introducing the vector constructs described herein into the cells by techniques readily available to the person of ordinary skill in the art. These include, but are not limited to, calcium phosphate transfection, DEAE-dextran-mediated transfection, cationic lipid-
20 mediated transfection, electroporation, transduction, infection, lipofection, and other techniques such as those found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual, 2nd, ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Host cells can contain more than one vector. Thus, different nucleotide
25 sequences can be introduced on different vectors of the same cell. Similarly, the polynucleotides of the invention can be introduced either alone or with other polynucleotides that are not related to the polynucleotides of the invention such as those providing trans-acting factors for expression vectors. When more than one vector is introduced into a cell, the vectors can be introduced independently, co-
30 introduced or joined to the polynucleotide vector.

In the case of bacteriophage and viral vectors, these can be introduced into cells as packaged or encapsulated virus by standard procedures for infection and

transduction. Viral vectors can be replication-competent or replication-defective. In the case in which viral replication is defective, replication will occur in host cells providing functions that complement the defects.

5 Vectors generally include selectable markers that enable the selection of the subpopulation of cells that contain the recombinant vector constructs. The marker can be contained in the same vector that contains the polynucleotides described herein or may be on a separate vector. Markers include tetracycline or ampicillin-resistance genes for prokaryotic host cells and dihydrofolate reductase or neomycin resistance for eukaryotic host cells. However, any marker that
10 provides selection for a phenotypic trait will be effective.

While the mature proteins can be produced in bacteria, yeast, mammalian cells, and other cells under the control of the appropriate regulatory sequences, cell-free transcription and translation systems can also be used to produce these proteins using RNA derived from the DNA constructs described herein.

15 Where secretion of the polypeptide is desired, appropriate secretion signals are incorporated into the vector. The signal sequence can be endogenous to the polypeptides of the invention or heterologous to these polypeptides.

Where the polypeptide is not secreted into the medium, the protein can be isolated from the host cell by standard disruption procedures, including freeze
20 thaw, sonication, mechanical disruption, use of lysing agents and the like. The polypeptide can then be recovered and purified by well-known purification methods including ammonium sulfate precipitation, acid extraction, anion or cationic exchange chromatography, phosphocellulose chromatography, hydrophobic-interaction chromatography, affinity chromatography,
25 hydroxylapatite chromatography, lectin chromatography, or high performance liquid chromatography.

It is also understood that depending upon the host cell in recombinant production of the polypeptides described herein, the polypeptides can have various glycosylation patterns, depending upon the cell, or maybe non-glycosylated as
30 when produced in bacteria. In addition, the polypeptides may include an initial modified methionine in some cases as a result of a host-mediated process.

Uses of vectors and host cells

It is understood that "host cells" and "recombinant host cells" refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

10 The host cells expressing the polypeptides described herein, and particularly recombinant host cells, have a variety of uses. First, the cells are useful for producing proteins or polypeptides of the invention that can be further purified to produce desired amounts of the protein or fragments. Thus, host cells containing expression vectors are useful for polypeptide production.

15 Host cells are also useful for conducting cell-based assays involving the protein or fragments. Thus, a recombinant host cell expressing a native protein of the invention is useful to assay for compounds that stimulate or inhibit protein function. This includes ligand binding, gene expression at the level of transcription or translation, G-protein interaction, and components of the signal transduction pathway.

20 Host cells are also useful for identifying mutants in which these functions are affected. If the mutants naturally occur and give rise to a pathology, host cells containing the mutations are useful to assay compounds that have a desired effect on the mutant protein (for example, stimulating or inhibiting function) which may not be indicated by their effect on the native protein.

25 Recombinant host cells are also useful for expressing the chimeric polypeptides described herein to assess compounds that activate or suppress activation by means of a heterologous amino terminal extracellular domain (or other binding region). Alternatively, a heterologous region spanning the entire transmembrane domain (or parts thereof) can be used to assess the effect of a desired amino terminal extracellular domain (or other binding region) on any given host cell. In this embodiment, a region spanning the entire transmembrane

domain (or parts thereof) compatible with the specific host cell is used to make the chimeric vector. Alternatively, a heterologous carboxy terminal intracellular, e.g., signal transduction, domain can be introduced into the host cell.

Further, mutant proteins can be designed in which one or more of the various functions is engineered to be increased or decreased (e.g., ligand binding or G-protein binding) and used to augment or replace the native proteins in an individual. Thus, host cells can provide a therapeutic benefit by replacing an aberrant protein of the invention or providing an aberrant protein that provides a therapeutic result. In one embodiment, the cells provide proteins that are abnormally active.

In another embodiment, the cells provide proteins that are abnormally inactive. These proteins can compete with the endogenous proteins in the individual.

In another embodiment, cells expressing proteins that cannot be activated, are introduced into an individual in order to compete with the endogenous proteins for ligand. For example, in the case in which excessive ligand is part of a treatment modality, it may be necessary to inactivate this ligand at a specific point in treatment. Providing cells that compete for the ligand, but which cannot be affected by receptor activation would be beneficial.

Homologously recombinant host cells can also be produced that allow the *in situ* alteration of the endogenous polynucleotide sequences in a host cell genome. The host cell includes, but is not limited to, a stable cell line, cell *in vivo*, or cloned microorganism. This technology is more fully described in WO 93/09222, WO 91/12650, WO 91/06667, U.S. 5,272,071, and U.S. 5,641,670.

Briefly, specific polynucleotide sequences corresponding to the polynucleotides or sequences proximal or distal to a gene of the invention are allowed to integrate into a host cell genome by homologous recombination where expression of the gene can be affected. In one embodiment, regulatory sequences are introduced that either increase or decrease expression of an endogenous sequence.

Accordingly, a protein of the invention can be produced in a cell not normally producing it. Alternatively, increased expression of the protein can be effected in a cell normally producing the protein at a specific level. Further, expression can

be decreased or eliminated by introducing a specific regulatory sequence. The regulatory sequence can be heterologous to the protein sequence or can be a homologous sequence with a desired mutation that affects expression.

Alternatively, the entire gene can be deleted. The regulatory sequence can be specific to the host cell or capable of functioning in more than one cell type. Still further, specific mutations can be introduced into any desired region of the gene to produce mutant proteins. Such mutations could be introduced, for example, into the specific functional regions such as the ligand-binding site.

In one embodiment, the host cell can be a fertilized oocyte or embryonic stem cell that can be used to produce a transgenic animal containing the altered gene. Alternatively, the host cell can be a stem cell or other early tissue precursor that gives rise to a specific subset of cells and can be used to produce transgenic tissues in an animal. See also Thomas *et al.*, *Cell* 51:503 (1987) for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced gene has homologously recombined with the endogenous receptor gene is selected (see e.g., Li, E. *et al.*, *Cell* 69:915 (1992)). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A.. (1991) *Current Opinions in Biotechnology* 2:823-829 and in PCT International Publication Nos. WO 90/11354; WO 91/01140; and WO 93/04169.

The genetically engineered host cells can be used to produce non-human transgenic animals. A transgenic animal is preferably a mammal, for example a rodent, such as a rat or mouse, in which one or more of the cells of the animal include a transgene. A transgene is exogenous DNA which is integrated into the

genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal in one or more cell types or tissues of the transgenic animal. These animals are useful for studying the function of a receptor protein and identifying and evaluating modulators of the protein activity.

5 Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, and amphibians.

In one embodiment, a host cell is a fertilized oocyte or an embryonic stem cell into which the polynucleotide sequences have been introduced.

10 A transgenic animal can be produced by introducing nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Any of the nucleotide sequences of the invention can be introduced as a transgene into the genome of a non-human animal, such as a mouse.

15 Any of the regulatory or other sequences useful in expression vectors can form part of the transgenic sequence. This includes intronic sequences and polyadenylation signals, if not already included. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of the protein to particular cells.

20 Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production
25 of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the transgene in its genome and/or expression of transgenic mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene can further be bred to other transgenic animals
30 carrying other transgenes. A transgenic animal also includes animals in which the entire animal or tissues in the animal have been produced using the homologously recombinant host cells described herein.

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g.,
5 Lakso *et al.* *PNAS* 89:6232-6236 (1992). Another example of a recombinase system is the FLP recombinase system of *S. cerevisiae* (O'Gorman *et al.* *Science* 251:1351-1355 (1991)). If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein is required. Such animals can be provided
10 through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* *Nature* 385:810-
15 813 (1997) and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is
20 isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

Transgenic animals containing recombinant cells that express the
25 polypeptides described herein are useful to conduct the assays described herein in an *in vivo* context. Accordingly, the various physiological factors that are present *in vivo* and that could effect ligand binding, receptor activation, and signal transduction, may not be evident from *in vitro* cell-free or cell-based assays. Accordingly, it is useful to provide non-human transgenic animals to assay *in vivo*
30 receptor function; including ligand interaction, the effect of specific mutant receptors on receptor function and ligand interaction, and the effect of chimeric

receptors. It is also possible to assess the effect of null mutations, that is mutations that substantially or completely eliminate one or more receptor functions.

In general, methods for producing transgenic animals include introducing a nucleic acid sequence according to the present invention, the nucleic acid sequence capable of expressing the protein in a transgenic animal, into a cell in culture or *in vivo*. When introduced *in vivo*, the nucleic acid is introduced into an intact organism such that one or more cell types and, accordingly, one or more tissue types, express the nucleic acid encoding the protein. Alternatively, the nucleic acid can be introduced into virtually all cells in an organism by transfecting a cell in culture, such as an embryonic stem cell, as described herein for the production of transgenic animals, and this cell can be used to produce an entire transgenic organism. As described, in a further embodiment, the host cell can be a fertilized oocyte. Such cells are then allowed to develop in a female foster animal to produce the transgenic organism.

15

Pharmaceutical compositions

The nucleic acid molecules of the invention, protein of the invention (particularly fragments such as the amino terminal extracellular domain), modulators of the protein, and antibodies (also referred to herein as "active compounds") can be incorporated into pharmaceutical compositions suitable for administration to a subject, e.g., a human. Such compositions typically comprise the nucleic acid molecule, protein, modulator, or antibody and a pharmaceutically acceptable carrier.

As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route

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of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the

injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

5 Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a seven-transmembrane protein/receptor protein or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the
10 preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier.
15 They can be enclosed in gelatin capsules or compressed into tablets. For oral administration, the agent can be contained in enteric forms to survive the stomach or further coated or mixed to be released in a particular region of the GI tract by known methods. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets,
20 troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of
25 the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such
30 as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the

dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

5 The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. 5,328,470) or by stereotactic injection (see e.g., Chen *et al.*, *PNAS* 91:3054-3057 (1994)). The pharmaceutical preparation of the gene therapy vector can include
10 the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

15 The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

 As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1
20 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

 The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or
25 age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for
30 between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or

polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low

dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

This invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will fully convey the invention to those skilled in the art. Many modifications and other embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Although specific terms are employed, they are used as in the art unless otherwise indicated.

Applicant's or agent's file reference	35800/207180	International application No.	1
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 7, line 16	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Blvd. Manassas, VA 20110-2209 US	
Date of deposit 06 April 2000 (06.04.00)	Accession Number PTA-1654
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Page 17, line 6; page 114, lines 8,12,16,20,24,28; page 115, lines 5,10,23,27,31; page 116, lines 4,8,12,25, 29; page 117, line 2,6,10,14,23,26,30; page 118, lines 11,17,27; page 119, lines 9,12,16,29,32; page 120, lines 4,22,25,29; page 121, lines 20,24,28,32; page 122, lines 4,8,26,30; page 123, lines 2,6,10,14,24,27,31; page 124, lines 23,26,30; page 125, lines 15,18,22; page 126, lines 6,10,14,18,22,26; page 127, lines 9,13,17,21,25,29; page 128, lines 11,14,18	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indicators are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 7, line 12	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depository institution American Type Culture Collection	
Address of depository institution (including postal code and country) 10801 University Blvd. Manassas, VA 20110-2209 US	
Date of deposit 09 May 2000 (09.05.00)	Accession Number PTA-1847
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Page 17, line 2; page 114, lines 8,12,16,21,24,29; page 115, lines 5,10,23,27,31; page 116, lines 5,8,13,25, 29; page 117, line 2,7,10,15,23,26,30; page 118, lines 11,16,22; page 119, lines 9,12,16,29,32; page 120, lines 4,22,25,29; page 121, lines 20,24,28; page 122, lines 1,4,9,26,30; page 123, lines 2,7,10,15,24,27,31; page 124, lines 23,26,30; page 125, lines 15,18,22; page 126, lines 6,10,14,19,22,27; page 127, lines 9,13,17,22,25,30; page 128, lines 11,14,18	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indicators are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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<input type="checkbox"/> This sheet was received with the International Bureau on:
Authorized officer

THAT WHICH IS CLAIMED:

1. An isolated nucleic acid molecule selected from the group consisting of:

- 5 a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
- 10 b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
- 15 c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;
- 20 d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847; and
- 25 e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a
- 30 nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule is selected from the group consisting of:

a) a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof; and

b) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847.

3. The nucleic acid molecule of claim 1, further having vector nucleotide sequences.

4. The nucleic acid molecule of claim 1, further having a nucleotide sequence encoding at least one heterologous polypeptide.

5. A host cell that contains a nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

5 d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847; and

10 e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions.

15

6. The host cell of claim 5, wherein said host cell is a mammalian host cell.

20 7. A nonhuman mammalian host cell containing at least one nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

25

b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

30 c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence

encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

5 d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit
10 Number PTA-1654 or PTA-1847; and

 e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-
15 1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions.

8. An isolated polypeptide selected from the group consisting of:

20 a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid
25 sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

 b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited
30 with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a

nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

- 5 c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof.

9. The isolated polypeptide of claim 8, wherein said polypeptide has an amino acid sequence selected from the group consisting of:

- 10 (a) SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847.

 (b) The amino acid sequence set forth as about amino acid 6 to about amino acid 337 of SEQ ID NO:1 or SEQ ID NO:3;

- 15 (c) The amino acid sequence extending from about amino acid 6 to about amino acid 337 of the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1847 or PTA-1654;

 (d) The amino acid sequence set forth as about amino acid 1 to about amino acid 37 of SEQ ID NO:1;

- 20 (e) The amino acid sequence extending from about amino acid 1 to about amino acid 37 of the polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-1847;

 (f) The amino acid sequence set forth as about amino acid 1 to about amino acid 40 of SEQ ID NO:3;

- 25 (g) The amino acid sequence extending from about amino acid 1 to about amino acid 40 of the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-1654; and

- 30 (h) The amino acid set forth as about amino acid 6 to about amino acid 450 of SEQ ID NO:5.

10. The polypeptide of claim 8, further having heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide, wherein said polypeptide is selected from the group consisting of:

a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof.

12. A method for producing a polypeptide selected from the group consisting of:

a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

- 5 b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6; or a complement thereof under stringent conditions; and
- 10 c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof;
- said method comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. The method of claim 12, wherein said polypeptide has the amino acid sequence of SEQ ID NO:1, 3, or 5.

14. A method for detecting the presence of a polypeptide in a sample, wherein said polypeptide is selected from the group consisting of:

- 20 a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;
- 25 b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and
- 30

- c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof;
- said method having the steps of contacting the sample with a compound that
- 5 selectively binds to the polypeptide and determining whether the compound binds to the polypeptide in the sample.

15. The method of claim 14, wherein the compound that binds to the polypeptide is an antibody.

10

16. A kit having a compound that selectively binds to a polypeptide of claim 8 and instructions for use.

17. A method for detecting the presence of a nucleic acid molecule in a

15 sample, wherein said nucleic acid molecule is selected from the group consisting of:

a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent

20 Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent

25 Deposit Number PTA-1654 or PTA-1847, or a complement thereof;

c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

d) a nucleic acid molecule encoding a fragment of a

30 polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or

PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847; and

- 5 e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a
10 nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions;

said method having the steps of contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the
15 sample.

18. The method of claim 17, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

20 19. A kit having a compound which selectively hybridizes to a nucleic acid molecule and instructions for use, wherein the nucleic acid molecule is selected from the group consisting of:

- a) a nucleic acid molecule having a nucleotide sequence that
 is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6,
25 the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
- b) a nucleic acid molecule having a fragment of at least 15
 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or
 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent
30 Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
- c) a nucleic acid molecule encoding a polypeptide having the
 amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence

encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

5 d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit
10 Number PTA-1654 or PTA-1847; and

e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-
15 1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions.

20. A method for identifying a compound which binds to a
20 polypeptide, wherein said polypeptide is selected from the group consisting of:

a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at
25 least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid
30 sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a

nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof;

said method having the steps of contacting the polypeptide or a cell expressing the polypeptide with a test compound and determining whether the polypeptide binds to the test compound.

21. The method of claim 20, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay;

c) detection of binding using an assay for GPCR-ligand binding.

22. A method for modulating the activity of a polypeptide selected from the group consisting of:

a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a

nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

- c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof;

said method having the steps of contacting the polypeptide or a cell expressing the polypeptide with a compound that binds to the polypeptide, under conditions in which the compound is capable of modulating the activity of the polypeptide.

23. A method for identifying a compound which modulates the activity of a polypeptide selected from the group consisting of:

- a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

- b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

- c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof;

said method having the steps of contacting the polypeptide with a test compound and determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

24. A method for identifying an agent that modulates the level of expression of a nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
 - b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
 - c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;
 - d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847; and
 - e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions;
- said method having the steps of contacting said agent with a cell expressing said nucleic acid molecule, under conditions such that said level of expression of said

nucleic acid molecule can be modulated in said cell by said agent; and measuring the level of expression of said nucleic acid molecule.

25. A method for modulating the level of expression of a nucleic acid molecule selected from the group consisting of:
- a) a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
 - b) a nucleic acid molecule having a fragment of at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:2, 4, or 6, the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, or a complement thereof;
 - c) a nucleic acid molecule encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;
 - d) a nucleic acid molecule encoding a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847; and
 - e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule having SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions;

said method comprising contacting said nucleic acid molecule with an agent under conditions that allow the agent to modulate the level of expression of the nucleic acid molecule.

5 26. A pharmaceutical composition containing at least one polypeptide in a pharmaceutically acceptable carrier, wherein said polypeptide is selected from the group consisting of:

10 a) a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the fragment has at least 12 contiguous amino acids of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847;

15 b) a naturally occurring allelic variant of a polypeptide having the amino acid sequence of SEQ ID NO:1, 3, or 5, or an amino acid sequence encoded by the cDNA insert of any of the plasmids deposited with ATCC as Patent Deposit Number PTA-1654 or PTA-1847, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleotide sequence having the nucleotide sequence set forth in SEQ ID NO:2, 4, or 6, or a complement thereof under stringent conditions; and

20 c) a polypeptide encoded by a nucleic acid molecule having a nucleotide sequence that is at least 60% identical to the nucleotide sequence of SEQ ID NO:2, 4, or 6, or a complement thereof.

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Sequence length 1729

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CTC ACC AGT TCG GAT GAA CTC AAT ACT ATT AAG TGG TAC AAC CTG ATT TTG ACT GCA ACT      615

T F C L P L V I V T L C Y T T I I H T L      225
ACT TTC TGC CTC CCC TTG GTG ATA GTG ACA CTT TGC TAT ACC ACG ATT ATC CAC ACT CTG      675

T H G L Q T D S C L K Q K A R R L T I L      245
ACC CAT GGA CTG CAA ACT GAC AGC TGC CTT AAG CAG AAA GCA CGA AGG CTA ACC ATT CTG      735

L L L A F Y V C F L P F H I L R V I R I      265
CTA CTC CTT GCA TTT TAC GTA TGT TTT TTA CCC TTC CAT ATC TTG AGG GTC ATT CGG ATC      795

E S R L L S I S C S I E N Q I H E A Y I      285
GAA TCT CGC CTG CTT TCA ATC AGT TGT TCC ATT GAG AAT CAG ATC CAT GAA GCT TAC ATC      855

V S G P L A A L N T F G N L L L Y V V V      305
GTT TCT GGA CCA TTA GCT GCT CTG AAC ACC TTT GGT AAC CTG TTA CTA TAT GTG GTG GTC      915

S D N F Q Q A V C S T V R C K V S G N L      325
AGC GAC AAC TTT CAG CAG GCT GTC TGC TCA ACA GTG AGA TGC AAA GTA AGC GGG AAC CTT      975

E Q A K K I S Y S N N P *      338

```

GAG CAA GCA AAG AAA ATT AGT TAA AAC AAC CCT TGA
AATATTTTCATTTACTTAACCAAAAACAAATACTTGCTGATACTTTACCTAGCATCCTAAGATGTTTCAGGATGTCCTCCCT
CAATGGAACCTCCTGGTAAATACTGIGTATTCAAGTAATCATGTGCCAAAGCCAGGGCAGAGCTTCTAGTTCTTTTGCAAT
CCCTTTATTGAGCTCCTCCACTGGGGAGATATAAGAATGGGATGCATGTATATCAGCAAAGTATTCAGACATAGTATTA
CAAGCTATTGGAACCTCAGAGGCATCTTAGAGAACATCTGTTCACCACTTACTATATATACAGGAAACCAATTTCT
TACCTTGCCCTAGATTGCTCAGTAAATTTGTGCCAAGATAGGAGAAAACCAATCTTTTCACTCATCATTTTCATGCTTC
TCTGCACTCTGGGCTATTGTATTGA

Analysis of 39404 (337 aa)

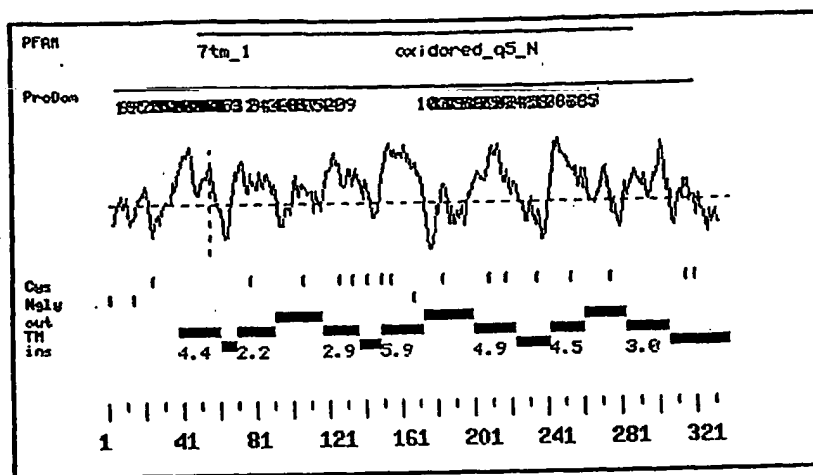


FIG 2

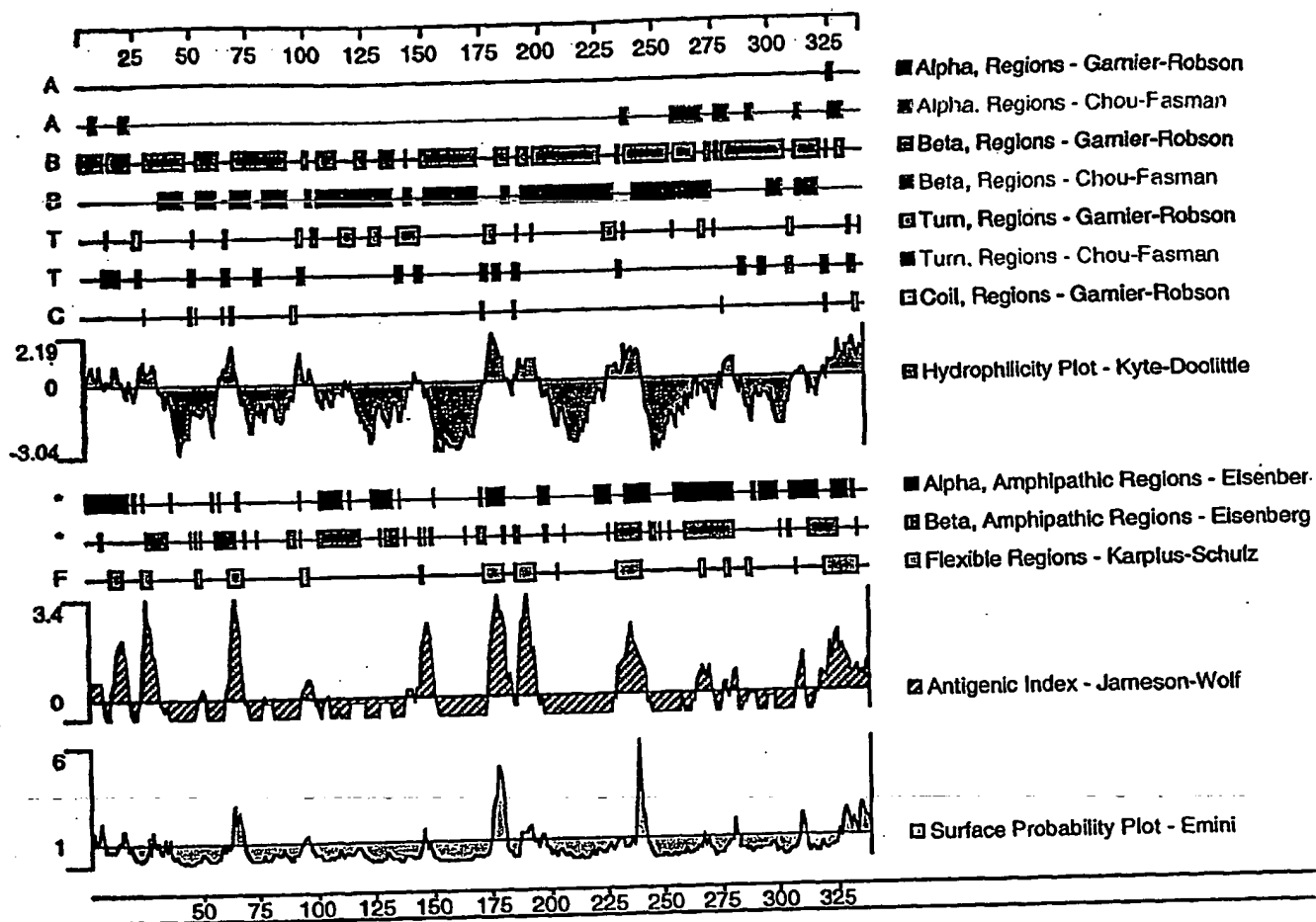


FIG 3

Method	Predict	Score	E _e
SignalP (eukaryote)	MAYBE		0.5

Signal Peptide Predictions td. 39404

Note: amino-terminal 70aa used for signal peptide prediction

Transmembrane Segments Predicted by MEMSAT

Start	End	Orient	Score
38	60	out->ins	4.4
70	90	ins->out	2.2
117	136	out->ins	2.9
149	172	ins->out	5.9
200	222	out->ins	4.9
242	260	ins->out	4.5
283	305	out->ins	3.0

Start	End	Orient	Score
16	36	ins->out	2.2
63	82	out->ins	2.9
95	118	ins->out	5.9
146	168	out->ins	4.9
188	206	ins->out	4.5
229	251	out->ins	3.0

Prosite Pattern Matches for 39404

Prosite version: Release 12.2 of February 1995

>PS00001|PDOC00001|ASN_GLYCOSYLATION N-glycosylation site.

Query: 10 NASD 13

Query: 23 NCTD 26

Query: 176 NRTN 179

>PS00004|PDOC00004|CAMP_PHOSPHO_SITE CAMP- and cGMP-dependent protein kinase phosphorylation site.

Query: 240 RRLT 243

Query: 329 KKIS 332

>PS00005|PDOC00005|PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.

Query: 175 TNR 177

Query: 178 TNR 180

Query: 194 TIK 196

Query: 316 TVR 318

>PS00006|PDOC00006|CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.

Query: 187 TSSD 190

Gene 39404 Expression in Normal Human Tissues

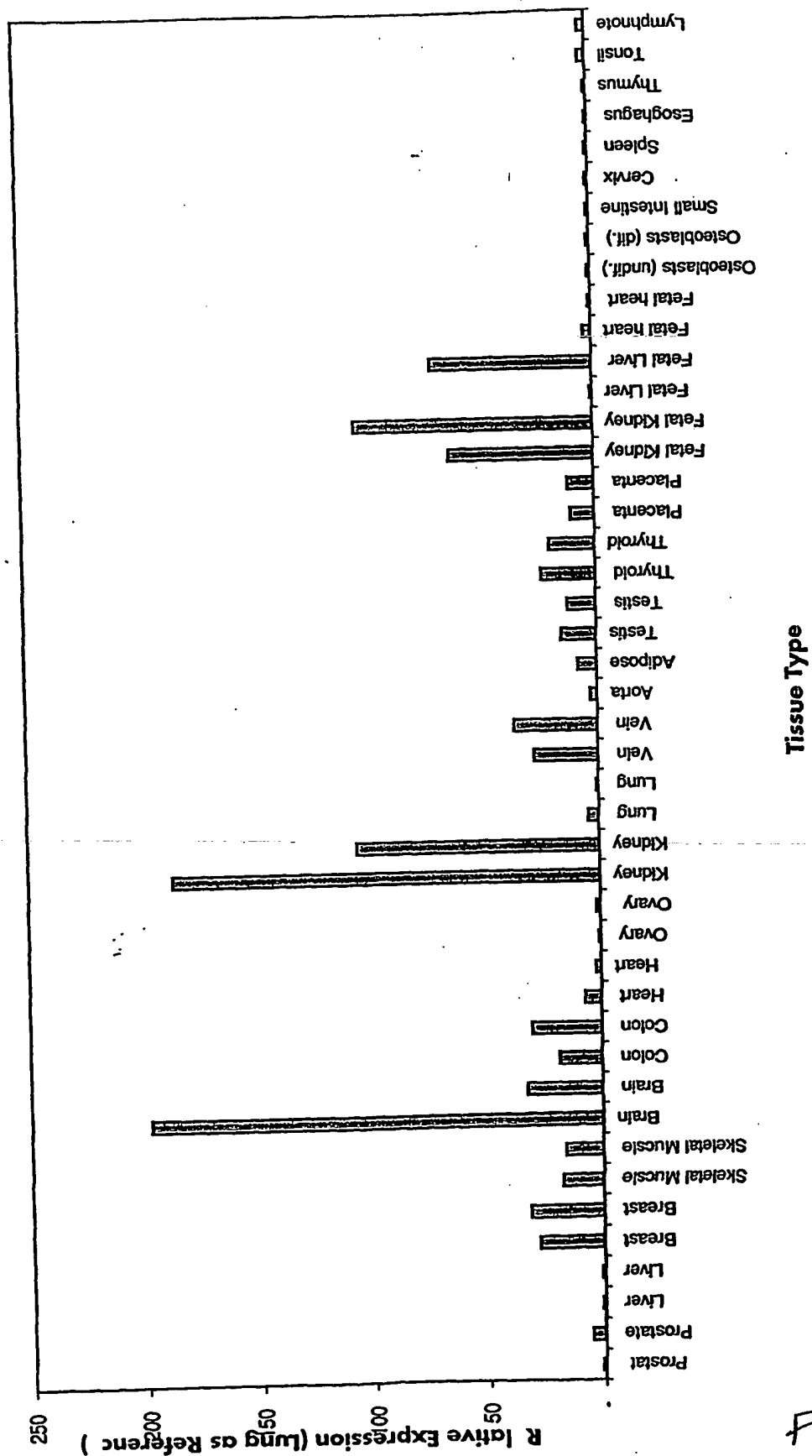


FIG 5

39404 Expression in Human CV Tissues

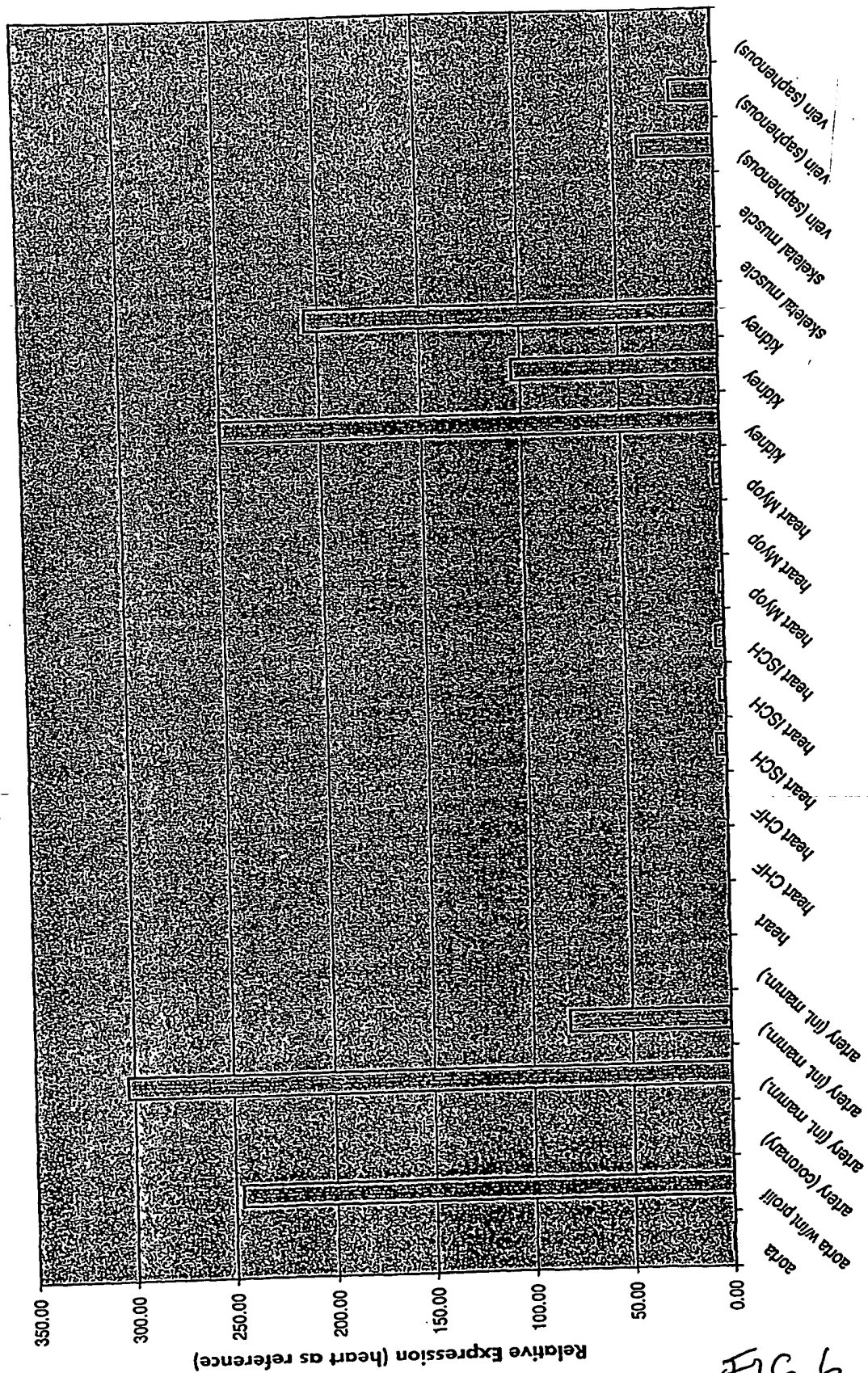


FIG 6

39404 Expression in Human CV tissues

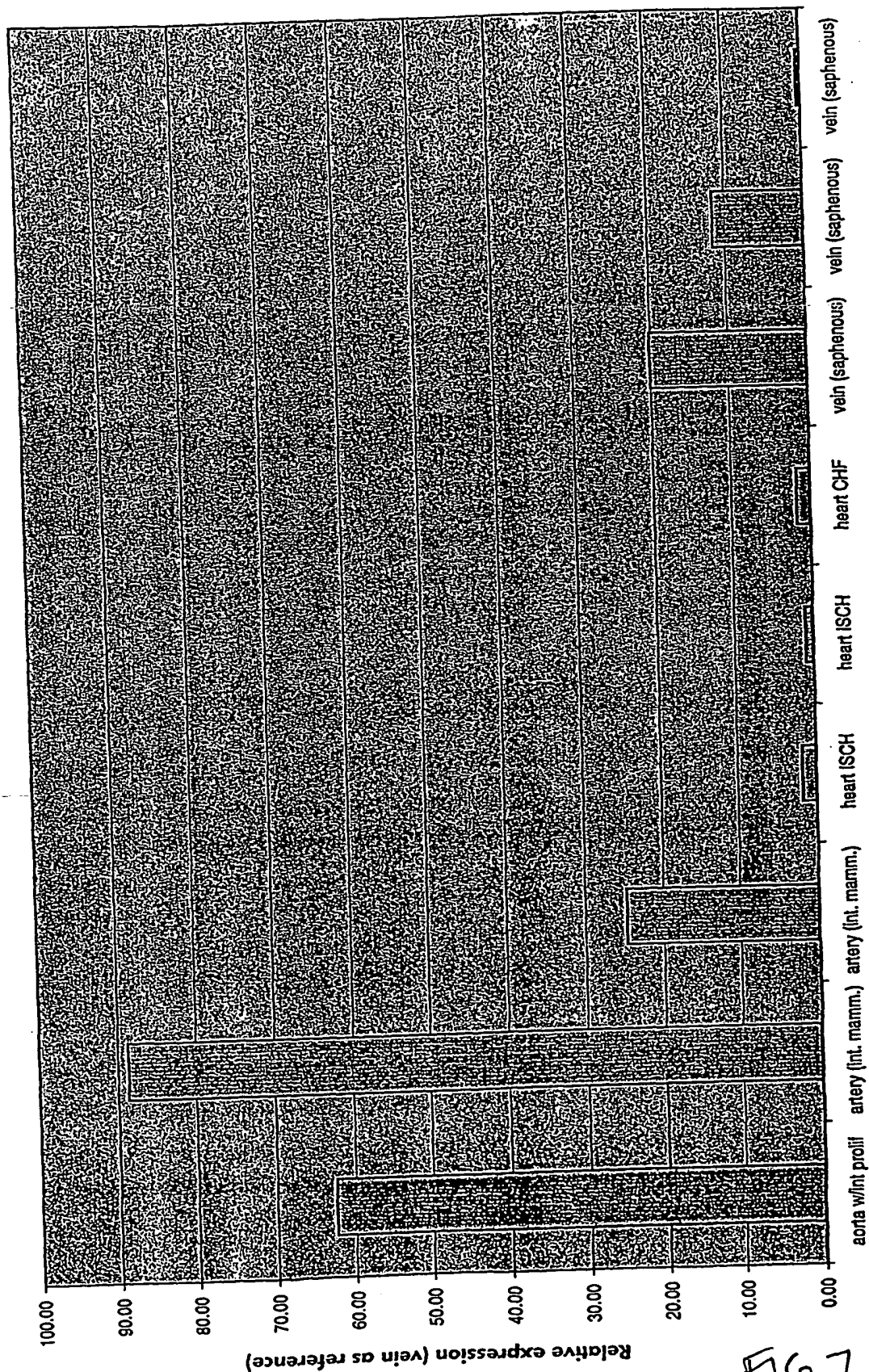


FIG 7

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Input file Fbh38911a.seq; Output file 38911.trans
Sequence length 1334

10	20	30	40	50	60	M	G	N	3											
GTC	GAC	GCT	GAC	AAATCTT	AACTCCTCA	AGGACTCC	CAAAACCAG	AGACACCAGGAGCCTGA	ATG GGG AAC	9										
D	S	V	S	Y	E	Y	G	D	Y	S	D	L	S	D	R	P	V	D	C	23
GAT	TCT	GTC	AGC	TAC	GAG	TAT	GGG	GAT	TAC	AGC	GAC	CTC	TCG	GAC	CGC	CCT	GTG	GAC	TGC	69
L	D	G	A	C	L	A	I	D	P	L	R	V	A	P	L	P	L	Y	A	43
CTG	GAT	GGC	GCC	TGC	CTG	GCC	ATC	GAC	CCG	CTG	CGC	GTG	GCC	CCG	CTC	CCA	CTG	TAT	GCC	129
A	I	F	L	V	G	V	P	G	N	A	M	V	A	W	V	A	G	K	V	63
GCC	ATC	TTC	CTG	GTG	GGG	GTG	CCG	GGC	AAT	GCC	ATG	GTG	GCC	TGG	GTG	GCT	GGG	AAG	GTG	189
A	R	R	R	V	G	A	T	W	L	L	H	L	A	V	A	D	L	L	C	83
GCC	CGC	CGG	AGG	GTG	GGT	GCC	ACC	TGG	TTG	CTC	CAC	CTG	GCC	GTG	GCG	GAT	TTG	CTG	TGC	249
C	L	S	L	P	I	L	A	V	P	I	A	R	G	G	H	W	P	Y	G	103
TGT	TTG	TCT	CTG	CCC	ATC	CTG	GCA	GTG	CCC	ATT	GCC	CGT	GGA	GGC	CAC	TGG	CCG	TAT	GGT	309
A	V	G	C	R	A	L	P	S	I	I	L	L	T	M	Y	A	S	V	L	123
GCA	GTG	GGC	TGT	CGG	GCG	CTG	CCC	TCC	ATC	ATC	CTG	CTG	ACC	ATG	TAT	GCC	AGC	GTC	CTG	369
L	L	A	A	L	S	A	D	L	C	F	L	A	L	G	P	A	W	W	S	143
CTC	CTG	GCA	GCT	CTC	AGT	GCC	GAC	CTC	TGC	TTT	CTG	GCT	CTC	GGG	CCT	GCC	TGG	TGG	TCT	429
T	V	Q	R	A	C	G	V	Q	V	A	C	G	A	A	W	T	L	A	L	163
ACG	GTT	CAG	CGG	GCG	TGC	GGG	GTG	CAG	GTG	GCC	TGT	GGG	GCA	GCC	TGG	ACA	CTG	GCC	TTG	489
L	L	T	V	P	S	A	I	Y	R	R	L	H	Q	E	H	F	P	A	R	183
CTG	CTC	ACC	GTG	CCC	TCC	GCC	ATC	TAC	CGC	CGG	CTG	CAC	CAG	GAG	CAC	TTC	CCA	GCC	CGG	549
L	Q	C	V	V	D	Y	G	G	S	S	S	T	E	N	A	V	T	A	I	203
CTG	CAG	TGT	GTG	GTG	GAC	TAC	GGC	GCC	TCC	TCC	AGC	ACC	GAG	AAT	GCG	GTG	ACT	GCC	ATC	609
R	F	L	F	G	F	L	G	P	L	V	A	V	A	S	C	H	S	A	L	223
CGG	TTT	CTT	TTT	GGC	TTC	CTG	GGG	CCC	CTG	GTG	GCC	GTG	GCC	AGC	TGC	CAC	AGT	GCC	CTC	669
L	C	W	A	A	R	R	C	R	P	L	G	T	A	I	V	V	G	F	F	243
CTG	TGC	TGG	GCA	GCC	CGA	CGC	TGC	CGG	CCG	CTG	GGC	ACA	GCC	ATT	GTG	GTG	GGG	TTT	TTT	729
V	C	W	A	P	Y	H	L	L	G	L	V	L	T	V	A	A	P	N	S	263
GTC	TGC	TGG	GCA	CCC	TAC	CAC	CTG	CTG	GGG	CTG	GTG	CTC	ACT	GTG	GCG	GCC	CCG	AAC	TCC	789
A	L	L	A	R	A	L	R	A	E	P	L	I	V	G	L	A	L	A	H	283
GCA	CTC	CTG	GCC	AGG	GCC	CTG	CGG	GCT	GAA	CCC	CTC	ATC	GTG	GCC	CTT	GCC	CTC	GCT	CAC	849
S	C	L	N	P	M	L	F	L	Y	F	G	R	A	Q	L	R	R	S	L	303
AGC	TGC	CTC	AAT	CCC	ATG	CTC	TTC	CTG	TAT	TTT	GGG	AGG	GCT	CAA	CTC	CGC	CGG	TCA	CTG	909
P	A	A	C	H	W	A	L	R	E	S	Q	G	Q	D	E	S	V	D	S	323
CCA	GCT	GCC	TGT	CAC	TGG	GCC	CTG	AGG	GAG	TCC	CAG	GGC	CAG	GAC	GAA	AGT	GTG	GAC	AGC	969
K	K	S	T	S	H	D	L	V	S	E	M	E	V	*						338
AAG	AAA	TCC	ACC	AGC	CAT	GAC	CTG	GTC	TCG	GAG	ATG	GAG	GTG	TAG						1014

GCTGGAGAGACATTGTGGGTGTGTATCTTCTTATCTCATTTCACAAGACTGGCTTCAGGCATAGCTGGATCCAGGAGCT
CAATGATGTCCTTCATTTTATTCCTTCCTTCATTCAACAGATATCCATCATGCACTTGCTATGTGCAAGGCTTTTITAGG

CACTAGAGATATAGCAGTGACCAAAACAG_____AAATCCTGCCCTCAGGGAGCTGATATTCTTCTAGTGGAGGA RCA
GACTATAAACAAAGATA

FIG 8 (CONT)

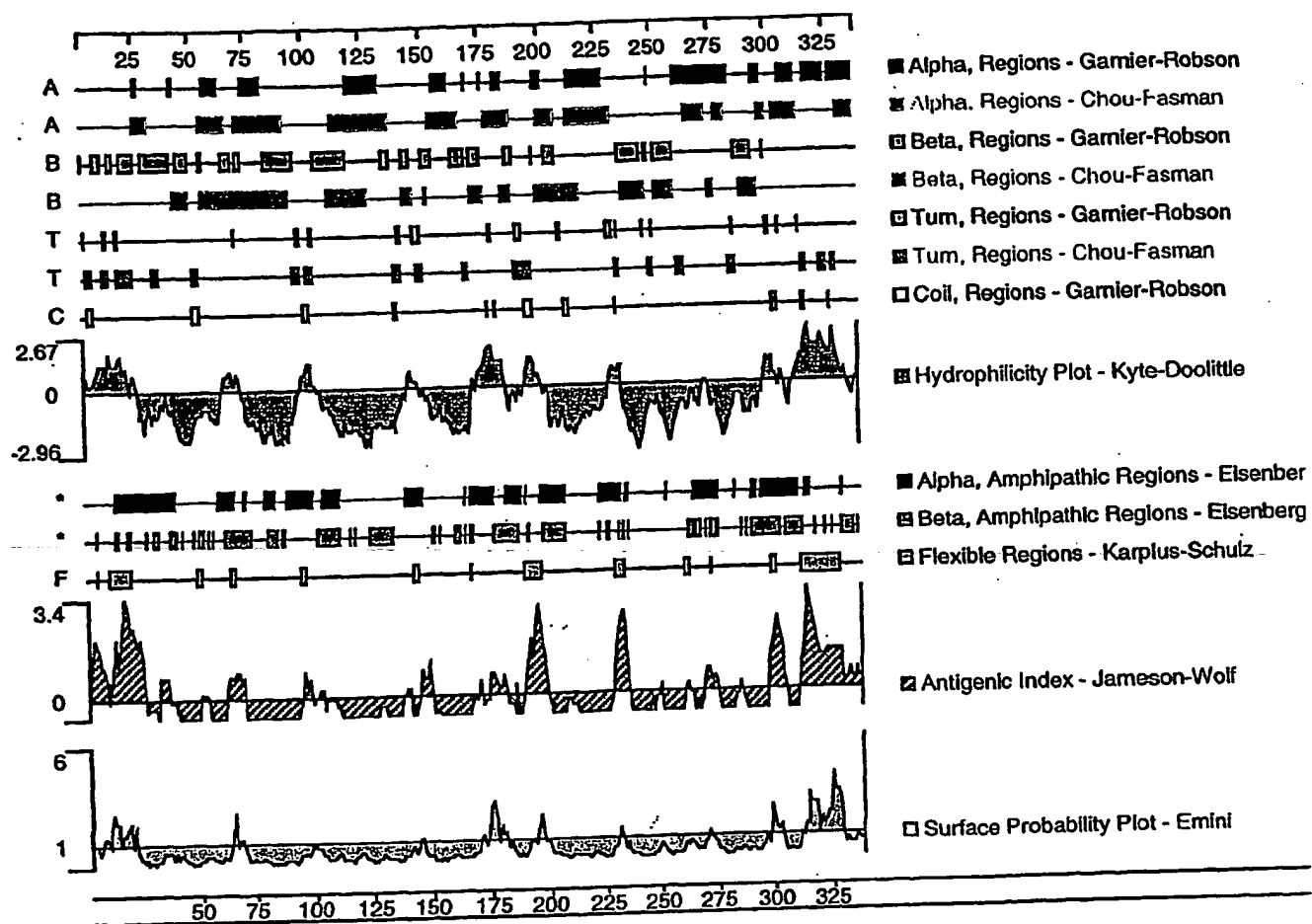


FIG 9

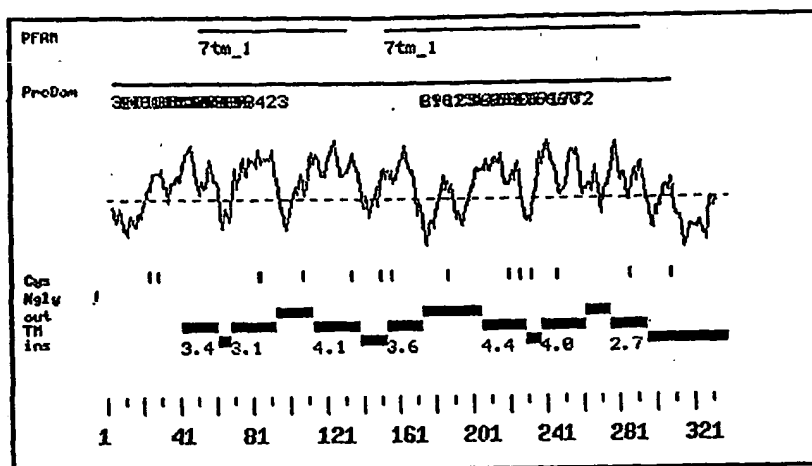
Analysis of 38911 (337 aa)

FIG 10

Transmembrane Segmer Predicted by MEMSAT

Start	End	Orient	Score
41	60	out->ins	3.4
68	92	ins->out	3.1
113	137	out->ins	4.1
153	172	ins->out	3.6
205	228	out->ins	4.4
237	260	ins->out	4.0
275	294	out->ins	2.7

Prosite Pattern Matches for 38911

>PS00001|PDOC00001|ASN_GLYCOSYLATION N-glycosylation site.

Query: 3 NDSV 6

>PS00004|PDOC00004|CAMP_PHOSPHO_SITE CAMP- and cGMP-dependent protein kinase phosphorylation site.

Query: 324 KKST 327

>PS00005|PDOC00005|PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.

Query: 17 SDR 19

Query: 323 SKK 325

>PS00006|PDOC00006|CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.

Query: 194 SSTE 197

Query: 327 TSHD 330

Query: 333 SEME 336

>PS00008|PDOC00008|MYRISTYL N-myristoylation site.

Query: 26 GACLA I. 31

Query: 49 GVPGNA 54

Query: 103 GAVGCR 108

Query: 150 GVQVAC 155

Query: 156 GAAWTL 161

Query: 191 GGSSST 196

Query: 253 GLVLTV 258

Query: 278 GLALAH 283

Query: 316 GQDESV 321

>PS00013|PDOC00013|PROKAR_LIPOPROTEIN Prokaryotic membrane lipoprotein lipid attachment site.

Non-eukaryotic pattern

RU Additional rules:

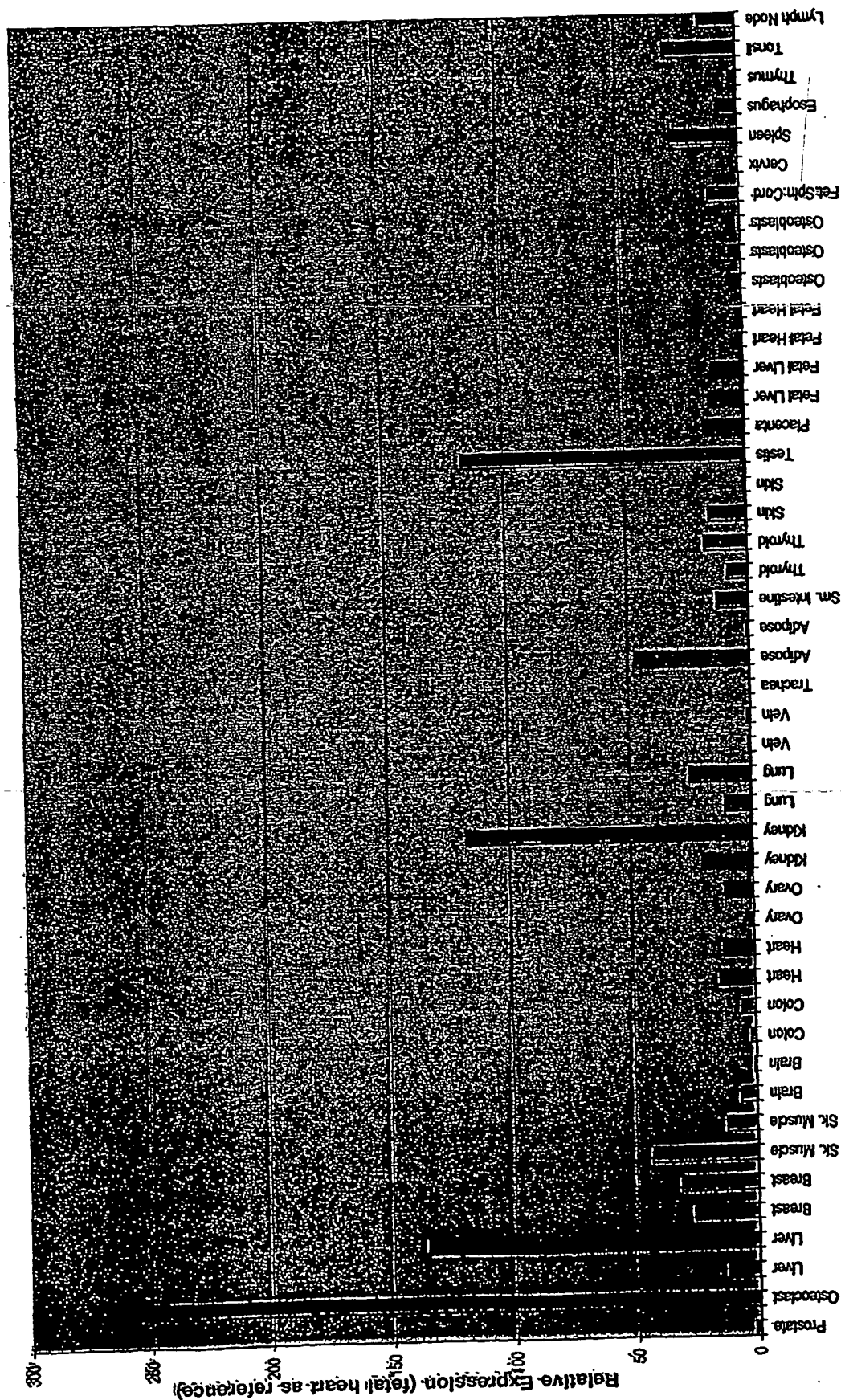
RU (1) The cysteine must be between positions 15 and 35 of the sequence in
 RU consideration.
 RU (2) There must be at least one charged residue (Lys or Arg) in the first
 RU seven residues of the sequence.

Query: 97 GGHWPYGA VGC 107

Query: 209 FLGPLVAVASC 219

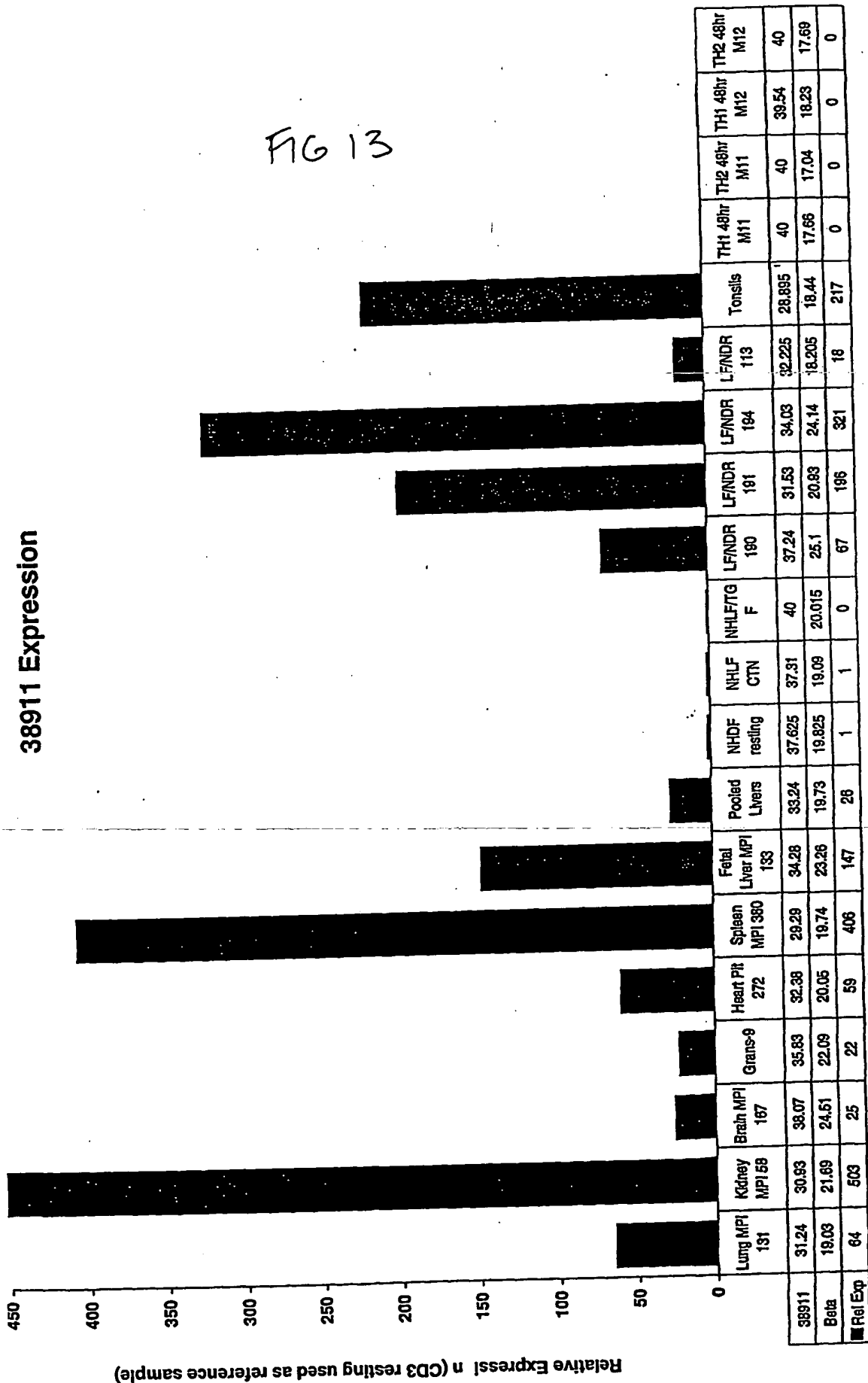
FIG 12

38911 expression in normal human tissues



38911 Expression

FIG 13



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Input file Fbh26904.seq: Input File 26904.trans
Sequence length 1743

10	20	30	40	50	60	70
80	90	100	110	120	130	140
150	160	170	180	190	200	210
220	230	240	250	260	270	280
290	300	310	320	330	340	350
360	370	380	390	400	410	420
430	440	450	460	470	480	490
500	510	520	530	540	550	560
570	580	590	600	610	620	630
640	650	660	670	680	690	700
710	720	730	740	750	760	770
780	790	800	810	820	830	840
850	860	870	880	890	900	910
920	930	940	950	960	970	980
990	1000	1010	1020	1030	1040	1050
1060	1070	1080	1090	1100	1110	1120
1130	1140	1150	1160	1170	1180	1190
1200	1210	1220	1230	1240	1250	1260
1270	1280	1290	1300	1310	1320	1330
1340	1350	1360	1370	1380	1390	1400
1410	1420	1430	1440	1450	1460	1470
1480	1490	1500	1510	1520	1530	1540
1550	1560	1570	1580	1590	1600	1610
1620	1630	1640	1650	1660	1670	1680
1690	1700	1710	1720	1730	1740	1750
1760	1770	1780	1790	1800	1810	1820
1830	1840	1850	1860	1870	1880	1890
1900	1910	1920	1930	1940	1950	1960
1970	1980	1990	2000	2010	2020	2030
2040	2050	2060	2070	2080	2090	2100
2110	2120	2130	2140	2150	2160	2170
2180	2190	2200	2210	2220	2230	2240
2250	2260	2270	2280	2290	2300	2310
2320	2330	2340	2350	2360	2370	2380
2390	2400	2410	2420	2430	2440	2450
2460	2470	2480	2490	2500	2510	2520
2530	2540	2550	2560	2570	2580	2590
2600	2610	2620	2630	2640	2650	2660
2670	2680	2690	2700	2710	2720	2730
2740	2750	2760	2770	2780	2790	2800
2810	2820	2830	2840	2850	2860	2870
2880	2890	2900	2910	2920	2930	2940
2950	2960	2970	2980	2990	3000	3010
3020	3030	3040	3050	3060	3070	3080
3090	3100	3110	3120	3130	3140	3150
3160	3170	3180	3190	3200	3210	3220
3230	3240	3250	3260	3270	3280	3290
3300	3310	3320	3330	3340	3350	3360
3370	3380	3390	3400	3410	3420	3430
3440	3450	3460	3470	3480	3490	3500
3510	3520	3530	3540	3550	3560	3570
3580	3590	3600	3610	3620	3630	3640
3650	3660	3670	3680	3690	3700	3710
3720	3730	3740	3750	3760	3770	3780
3790	3800	3810	3820	3830	3840	3850
3860	3870	3880	3890	3900	3910	3920
3930	3940	3950	3960	3970	3980	3990
4000	4010	4020	4030	4040	4050	4060
4070	4080	4090	4100	4110	4120	4130
4140	4150	4160	4170	4180	4190	4200
4210	4220	4230	4240	4250	4260	4270
4280	4290	4300	4310	4320	4330	4340
4350	4360	4370	4380	4390	4400	4410
4420	4430	4440	4450	4460	4470	4480
4490	4500	4510	4520	4530	4540	4550
4560	4570	4580	4590	4600	4610	4620
4630	4640	4650	4660	4670	4680	4690
4700	4710	4720	4730	4740	4750	4760
4770	4780	4790	4800	4810	4820	4830
4840	4850	4860	4870	4880	4890	4900
4910	4920	4930	4940	4950	4960	4970
4980	4990	5000	5010	5020	5030	5040
5050	5060	5070	5080	5090	5100	5110
5120	5130	5140	5150	5160	5170	5180
5190	5200	5210	5220	5230	5240	5250
5260	5270	5280	5290	5300	5310	5320
5330	5340	5350	5360	5370	5380	5390
5400	5410	5420	5430	5440	5450	5460
5470	5480	5490	5500	5510	5520	5530
5540	5550	5560	5570	5580	5590	5600
5610	5620	5630	5640	5650	5660	5670
5680	5690	5700	5710	5720	5730	5740
5750	5760	5770	5780	5790	5800	5810
5820	5830	5840	5850	5860	5870	5880
5890	5900	5910	5920	5930	5940	5950
5960	5970	5980	5990	6000	6010	6020
6030	6040	6050	6060	6070	6080	6090
6100	6110	6120	6130	6140	6150	6160
6170	6180	6190	6200	6210	6220	6230
6240	6250	6260	6270	6280	6290	6300
6310	6320	6330	6340	6350	6360	6370
6380	6390	6400	6410	6420	6430	6440
6450	6460	6470	6480	6490	6500	6510
6520	6530	6540	6550	6560	6570	6580
6590	6600	6610	6620	6630	6640	6650
6660	6670	6680	6690	6700	6710	6720
6730	6740	6750	6760	6770	6780	6790
6800	6810	6820	6830	6840	6850	6860
6870	6880	6890	6900	6910	6920	6930
6940	6950	6960	6970	6980	6990	7000
7010	7020	7030	7040	7050	7060	7070
7080	7090	7100	7110	7120	7130	7140
7150	7160	7170	7180	7190	7200	7210
7220	7230	7240	7250	7260	7270	7280
7290	7300	7310	7320	7330	7340	7350
7360	7370	7380	7390	7400	7410	7420
7430	7440	7450	7460	7470	7480	7490
7500	7510	7520	7530	7540	7550	7560
7570	7580	7590	7600	7610	7620	7630
7640	7650	7660	7670	7680	7690	7700
7710	7720	7730	7740	7750	7760	7770
7780	7790	7800	7810	7820	7830	7840
7850	7860	7870	7880	7890	7900	7910
7920	7930	7940	7950	7960	7970	7980
7990	8000	8010	8020	8030	8040	8050
8060	8070	8080	8090	8100	8110	8120
8130	8140	8150	8160	8170	8180	8190
8200	8210	8220	8230	8240	8250	8260
8270	8280	8290	8300	8310	8320	8330
8340	8350	8360	8370	8380	8390	8400
8410	8420	8430	8440	8450	8460	8470
8480	8490	8500	8510	8520	8530	8540
8550	8560	8570	8580	8590	8600	8610
8620	8630	8640	8650	8660	8670	8680
8690	8700	8710	8720	8730	8740	8750
8760	8770	8780	8790	8800	8810	8820
8830	8840	8850	8860	8870	8880	8890
8900	8910	8920	8930	8940	8950	8960
8970	8980	8990	9000	9010	9020	9030
9040	9050	9060	9070	9080	9090	9100
9110	9120	9130	9140	9150	9160	9170
9180	9190	9200	9210	9220	9230	9240
9250	9260	9270	9280	9290	9300	9310
9320	9330	9340	9350	9360	9370	9380
9390	9400	9410	9420	9430	9440	9450
9460	9470	9480	9490	9500	9510	9520
9530	9540	9550	9560	9570	9580	9590
9600	9610	9620	9630	9640	9650	9660
9670	9680	9690	9700	9710	9720	9730
9740	9750	9760	9770	9780	9790	9800
9810	9820	9830	9840	9850	9860	9870
9880	9890	9900	9910	9920	9930	9940
9950	9960	9970	9980	9990	10000	10010
10020	10030	10040	10050	10060	10070	10080
10090	10100	10110	10120	10130	10140	10150
10160	10170	10180	10190	10200	10210	10220
10230	10240	10250	10260	10270	10280	10290
10300	10310	10320	10330	10340	10350	10360
10370	10380	10390	10400	10410	10420	10430
10440	10450	10460	10470	10480	10490	10500
10510	10520	10530	10540	10550	10560	10570
10580	10590	10600	10610	10620	10630	10640
10650	10660	10670	10680	10690	10700	10710
10720	10730	10740	10750	10760	10770	10780
10790	10800	10810	10820	10830	10840	10850
10860	10870	10880	10890	10900	10910	10920
10930	10940	10950	10960	10970	10980	10990
11000	11010	11020	11030	11040	11050	11060
11070	11080	11090	11100	11110	11120	11130
11140	11150	11160	11170	11180	11190	11200
11210	11220	11230	11240	11250	11260	11270
11280	11290	11300	11310	11320	11330	11340
11350	11360	11370	11380	11390	11400	11410
11420	11430	11440	11450	11460	11470	11480
11490	11500	11510	11520	11530	11540	11550
11560	11570	11580	11590	11600	11610	11620
11630	11640	11650	11660	11670	11680	11690
11700	11710	11720	11730	11740	11750	11760
11770	11780	11790	11800	11810	11820	11830
11840	11850	11860	11870	11880	11890	11900
11910	11920	11930	11940	11950	11960	11970
11980	11990	12000	12010	12020	12030	12040
12050	12060	12070	12080	12090	12100	12110
12120	12130	12140	12150	12160	12170	12180
12190	12200	12210	12220	12230		

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E E K Y L K E A M E C S D V I W Q R G L					359	
GAG GAG AAG TAC TTG AAA GAG GCC ATG GAG TGT AGC GAT GTG ATT TGG CAG CGA GGT TTG					1077	123
L R K G Y G I C H G T A G H G Y S F L S					379	
CTG CGG AAG GGC TAC GGG ATA TGC CAT GGG ACT GCT GGC CAC GGC TAT TCC TTC CTG TCC					1137	129
L Y R L T Q D K K Y L Y R A C K F A E W					399	
CTT TAC CGT CTC ACG CAG GAT AAG AAG TAC CTC TAC CGA GCT TGC AAG TTT GCA GAG TGG					1197	135
C L D Y G A H G C R I P D R P Y S L F E					419	
TGT CTA GAT TAC GGA GCA CAC GGG TGC CGC ATT CCT GAC AGA CCC TAT TCG CTC TTT GAA					1257	141
G M A G A I H F L S D V L G P E T S R F					439	
GGC ATG GCT GGC GCT ATT CAC TTT CTC TCT GAT GTC CTG GGA CCA GAG ACA TCA CGG TTT					1317	147
P A F E L D S S K R D *					451	
CCA GCA TTT GAA CTT GAC TCT TCG AAG AGG GAT TAA					1353	151

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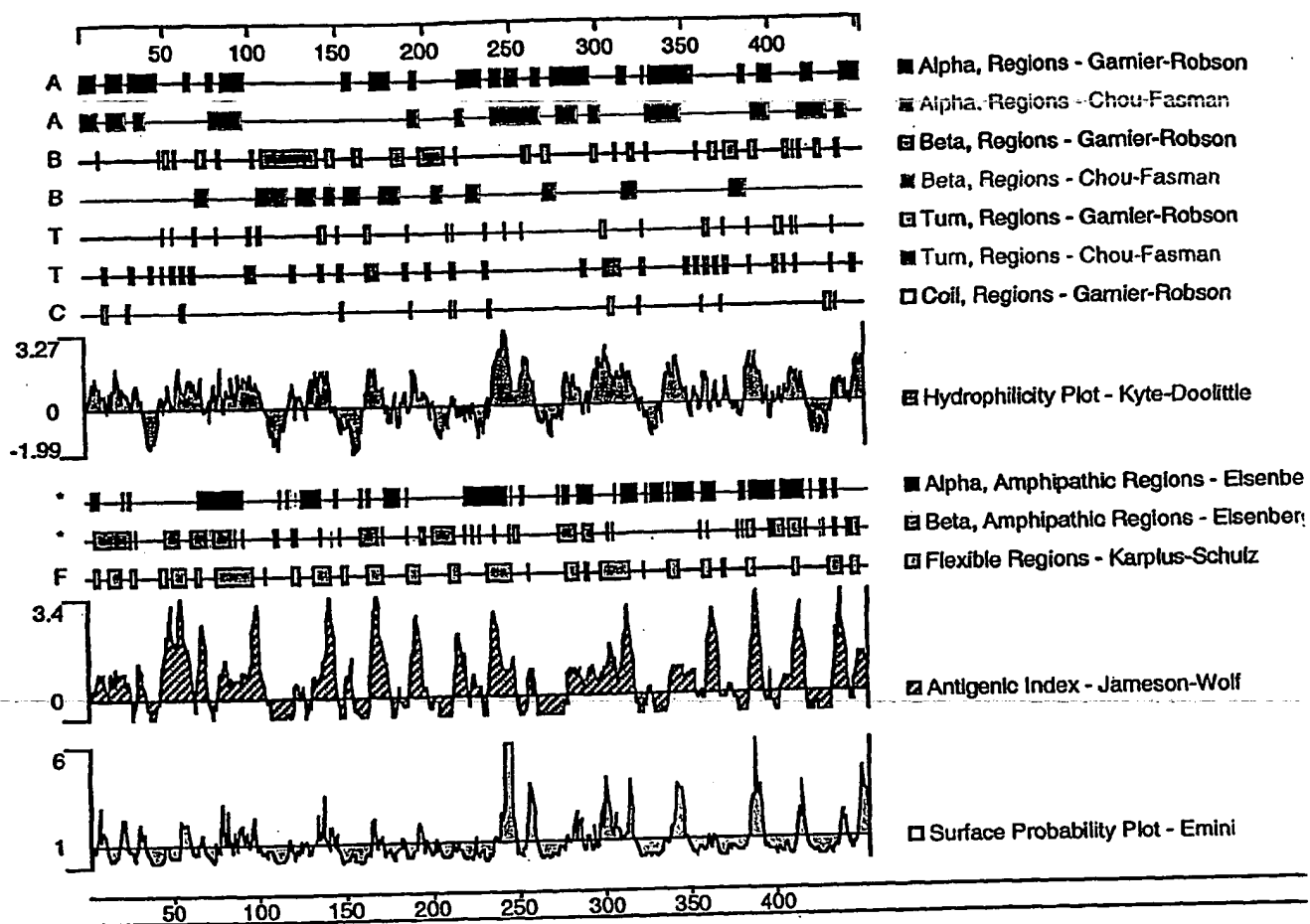


FIG 15

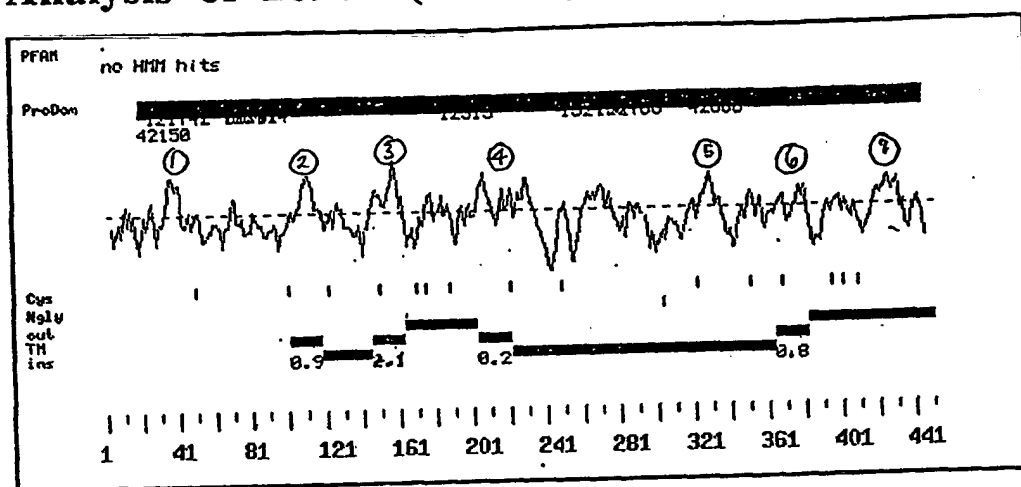
Analysis of 26904 (450 aa)

FIG 16

20/22

Transmembrane Segments Predicted by MEMSAT

Start	End	Orient	
101	117	out->ins	0.9
145	162	ins->out	2.1
203	220	out->ins	0.2
364	381	ins->out	0.8

Prosite Pattern Matches for 26904

>PS00001|PDOC00001|ASN_GLYCOSYLATION N-glycosylation site.

Query: 312 NETD 315

>PS00004|PDOC00004|CAMP_PHOSPHO_SITE cAMP- and cGMP-dependent protein kinase phosphorylation site.

Query: 143 RRVV 146

>PS00005|PDOC00005|PKC_PHOSPHO_SITE Protein kinase C phosphorylation site.

Query: 6 SKR 8

Query: 136 TLR 138

Query: 234 SGK 236

Query: 245 TER 247

Query: 314 TDR 316

Query: 436 TSR 438

Query: 446 SSK 448

>PS00006|PDOC00006|CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.

Query: 55 TTDE 58

Query: 167 SDCE 170

Query: 218 TVCE 221

Query: 239 SREE 242

Query: 284 TLTE 287

Query: 416 SLFE 419

Query: 447 SKRD 450

>PS00007|PDOC00007|TYR_PHOSPHO_SITE Tyrosine kinase phosphorylation site.

Query: 118 RVTCQTY 125

Query: 336 KVFKEKY 343

Query: 382 RLATQKY 389

Query: 409 RIFDRFY 415

>PS00008|PDOC00008|MYRISTYL N-myristoylation site.

Query: 36 GALLAS 41

Query: 91 OLKTAD 96

Query: 261 GAAGH 266

Query: 304 GNYPS 309

Query: 365 GICGT 370

Query: 404 GAHGR 409

Query: 420 GNAGAI 425

>PS00009|PDOC00009|AMIDATION Amidation site.

Query: 141 NGRR 144

>PS00011|PDOC00011|ATP_GTP_A ATP/GTP-binding site motif A (P-loop).

Query: 230 AIIESGKT 237

FIG 17

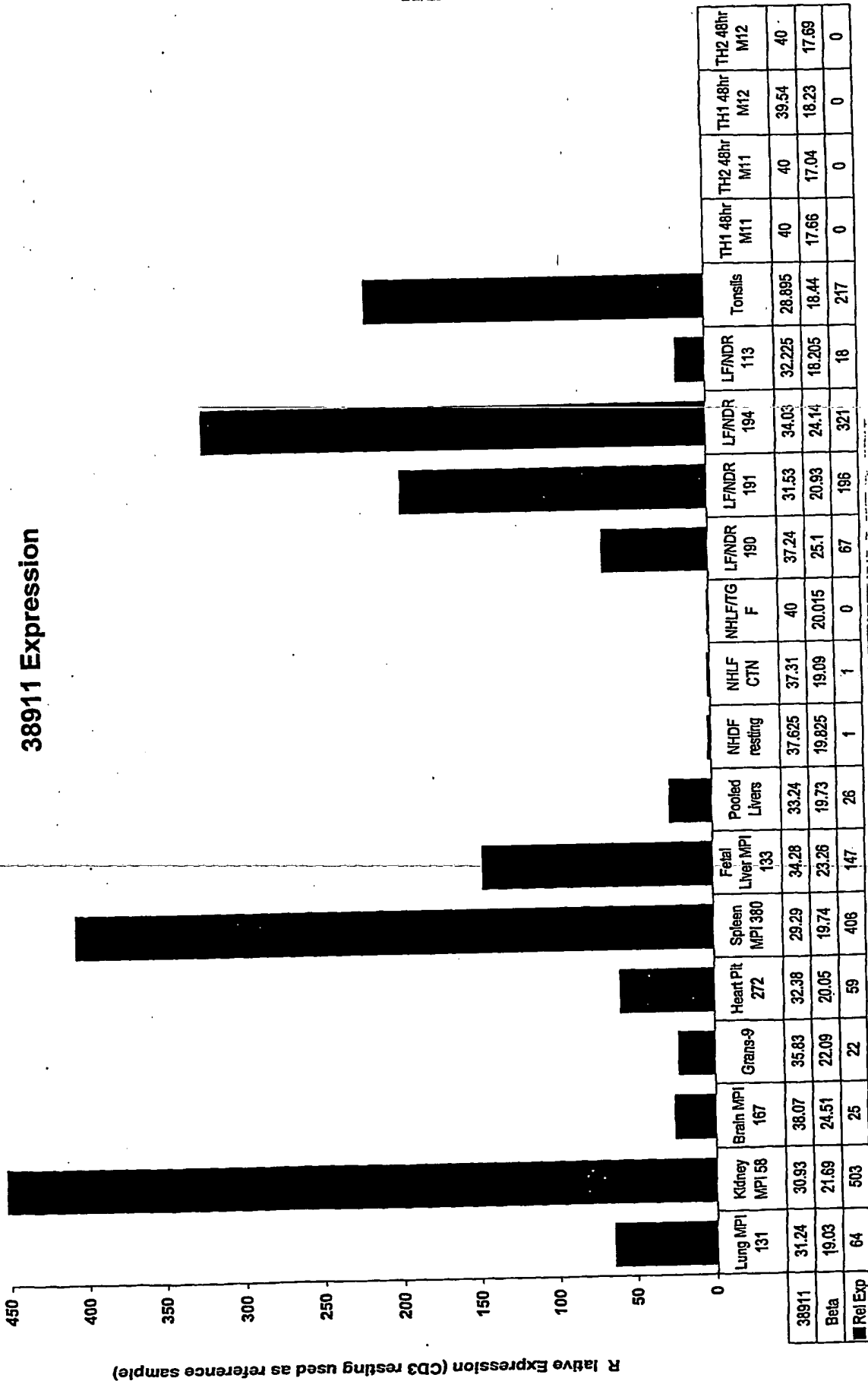


FIGURE 18

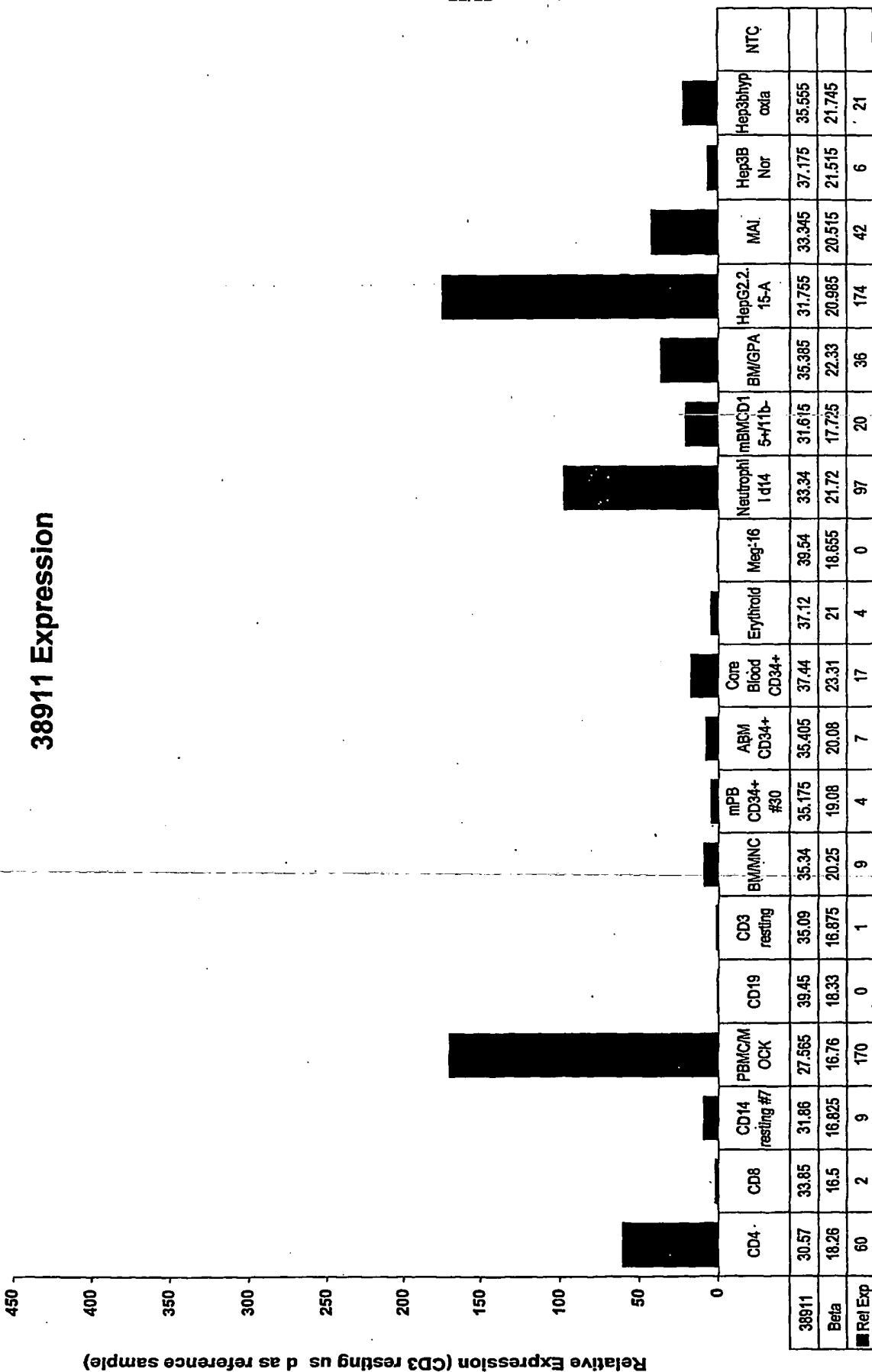


FIGURE 19

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White, David

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Seven-Transmembrane Proteins/G-Protein Coupled Receptors

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Met His Tyr Leu Pro Val Ile Tyr Gly Ile Ile Phe Leu Val Gly Phe
      35           40           45
Pro Gly Asn Ala Val Val Ile Ser Thr Tyr Ile Phe Lys Met Arg Pro
      50           55           60
Trp Lys Ser Ser Thr Ile Ile Met Leu Asn Leu Ala Cys Thr Asp Leu
      65           70           75           80
Leu Tyr Leu Thr Ser Leu Pro Phe Leu Ile His Tyr Tyr Ala Ser Gly
      85           90           95
Glu Asn Trp Ile Phe Gly Asp Phe Met Cys Lys Phe Ile Arg Phe Ser
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Phe His Phe Asn Leu Tyr Ser Ser Ile Leu Phe Leu Thr Cys Phe Ser
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His Lys Thr Arg Cys Ala Val Val Ala Cys Ala Val Val Trp Ile Ile
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Val Ser Asp Asn Phe Gln Gln Ala Val Cys Ser Thr Val Arg Cys Lys
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 gagaagtggc aaatttagga tgtagaata attttcattt aaaagtagat ccttggtttt 180
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 catattgcca aactgaactc tcttggttttc ttgcaagatg aaaggagaca acc atg 296
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 Ala Ala Ala Phe Gly Asn Cys Thr Asp Glu Asn Ile Pro Leu Lys Met
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 Gly Asn Ala Val Val Ile Ser Thr Tyr Ile Phe Lys Met Arg Pro Trp
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 Lys Ser Ser Thr Ile Ile Met Leu Asn Leu Ala Cys Thr Asp Leu Leu
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 Cys Ser Ile Glu Asn Gln Ile His Glu Ala Tyr Ile Val Ser Gly Pro
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 195 200 205
 Phe Leu Gly Pro Leu Val Ala Val Ala Ser Cys His Ser Ala Leu Leu
 210 215 220
 Cys Trp Ala Ala Arg Arg Cys Arg Pro Leu Gly Thr Ala Ile Val Val
 225 230 235 240
 Gly Phe Phe Val Cys Trp Ala Pro Tyr His Leu Leu Gly Leu Val Leu
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 260 265 270
 Glu Pro Leu Ile Val Gly Leu Ala Leu Ala His Ser Cys Leu Asn Pro
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 atc gac ccg ctg cgc gtg gcc ccg ctc cca ctg tat gcc gcc atc ttc 204
 Ile Asp Pro Leu Arg Val Ala Pro Leu Pro Leu Tyr Ala Ala Ile Phe
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 Val Ala Arg Arg Arg Val Gly Ala Thr Trp Leu Leu His Leu Ala Val
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 Ala Asp Leu Leu Cys Cys Leu Ser Leu Pro Ile Leu Ala Val Pro Ile
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 gcc cgt gga ggc cac tgg ccg tat ggt gca gtg ggc tgt cgg gcg ctg 396

Ala Arg Gly Gly His Trp Pro Tyr Gly Ala Val Gly Cys Arg Ala Leu	
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Pro Ser Ile Ile Leu Leu Thr Met Tyr Ala Ser Val Leu Leu Leu Ala	
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Val Val Gly Phe Phe Val Cys Trp Ala Pro Tyr His Leu Leu Gly Leu	
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Val Leu Thr Val Ala Ala Pro Asn Ser Ala Leu Leu Ala Arg Ala Leu	
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Arg Ala Glu Pro Leu Ile Val Gly Leu Ala Leu Ala His Ser Cys Leu	
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 Ala Ile Ile Glu Ser Gly Lys Thr Leu Ser Arg Glu Glu Arg Lys Thr
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 gag cgc tgc ccg ctg ttg tac cag tgg cac cgg aag cag tac gtt gga 944
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 His Gly Cys Arg Ile Pro Asp Arg Pro Tyr Ser Leu Phe Glu Gly Met
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 Ala Gly Ala Ile His Phe Leu Ser Asp Val Leu Gly Pro Glu Thr Ser
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(10) International Publication Number
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09/475,790 30 December 1999 (30.12.1999) US
- (71) Applicant (for all designated States except US): **MILLENNIUM PHARMACEUTICALS, INC.** [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US).
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- (74) Agents: **COULTER, Kathryn, L. et al.**; Alston & Bird LLP, Bank of America Plaza, Suite 4000, 101 South Tryon Street, Charlotte, NC 28280-4000 (US).
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- (88) Date of publication of the international search report:
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: 26904, 38911, AND 39404, SEVEN-TRANSMEMBRANE PROTEINS/G-PROTEIN COUPLED RECEPTORS

(57) Abstract: The present invention relates to seven-transmembrane proteins, including proteins that function as receptors belonging to the superfamily of G-protein-coupled receptors. The invention also relates to polynucleotides encoding the seven-transmembrane proteins/receptors. The invention further relates to methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides as a target for diagnosis and treatment in seven-transmembrane protein/receptor-mediated and related disorders. The invention further relates to drug-screening methods using the seven-transmembrane protein/receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the seven-transmembrane protein/receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the receptor polypeptides and polynucleotides.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/62 C12N15/11 C07K14/72 C07K19/00
C07K16/28 C12Q1/68 G01N33/50 G01N33/566 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBL, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 36473 A (PARODI LUIS A ;LIND PETER (SE); SEJLITZ TORSTEN (SE); SCHELLIN KAT) 25 May 2001 (2001-05-25) SEQ.IDs. 85 and 86 ---	1-26
E	WO 01 36471 A (ARENA PHARMACEUTICALS INC ;CHEN RUOPING (US); DANG HUONG T (US); L) 25 May 2001 (2001-05-25) SEQ.IDs. 27 and 28 ---	1-26
A	STADEL ET AL.: "Orphan G protein-coupled receptors:" TRENDS IN PHARMACOLOGICAL SCIENCES, no. 18, 1 November 1997 (1997-11-01), pages 430-437, XP002073279 ISSN: 0165-6147 the whole document -----	1-26

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 August 2001

Date of mailing of the international search report

31 10 2001

Name and mailing address of the ISA

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Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/35309

B x I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see extra sheet invention 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26 (all partially)

An isolated nucleic acid molecule having a nucleotide sequence that is at least 60% identical to SEQ.ID. 2 and fragments and allelic variants thereof; a host cell that contains said nucleic acid molecule; an isolated polypeptide encoded by said nucleic acid molecule and fragments thereof; an antibody which selectively binds to said polypeptide; a method for producing said polypeptide; methods for detecting the presence of said nucleic acid molecule or said polypeptide; kits having compounds that selectively bind to said polypeptide or hybridize to said nucleic acid; methods for identifying compounds which bind to said polypeptide or compounds which modulate the activity of said polypeptide; a method for modulating the activity of said polypeptide; a method for identifying an agent that modulates the level of expression of said nucleic acid molecule; a method for modulating the level of expression of said nucleic acid molecule; and a pharmaceutical composition containing said polypeptide.

2. Claims: 1-26 (all partially)

An isolated nucleic acid molecule having a nucleotide sequence that is at least 60% identical to SEQ.ID. 4 and fragments and allelic variants thereof; a host cell that contains said nucleic acid molecule; an isolated polypeptide encoded by said nucleic acid molecule and fragments thereof; an antibody which selectively binds to said polypeptide; a method for producing said polypeptide; methods for detecting the presence of said nucleic acid molecule or said polypeptide; kits having compounds that selectively bind to said polypeptide or hybridize to said nucleic acid; methods for identifying compounds which bind to said polypeptide or compounds which modulate the activity of said polypeptide; a method for modulating the activity of said polypeptide; a method for identifying an agent that modulates the level of expression of said nucleic acid molecule; a method for modulating the level of expression of said nucleic acid molecule; and a pharmaceutical composition containing said polypeptide.

3. Claims: 1-26 (all partially)

An isolated nucleic acid molecule having a nucleotide sequence that is at least 60% identical to SEQ.ID. 6 and fragments and allelic variants thereof; a host cell that contains said nucleic acid molecule; an isolated polypeptide encoded by said nucleic acid molecule and fragments thereof; an antibody which selectively binds to said polypeptide; a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

method for producing said polypeptide; methods for detecting the presence of said nucleic acid molecule or said polypeptide; kits having compounds that selectively bind to said polypeptide or hybridize to said nucleic acid; methods for identifying compounds which bind to said polypeptide or compounds which modulate the activity of said polypeptide; a method for modulating the activity of said polypeptide; a method for identifying an agent that modulates the level of expression of said nucleic acid molecule; a method for modulating the level of expression of said nucleic acid molecule; and a pharmaceutical composition containing said polypeptide.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/35309

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0136473	A	25-05-2001	AU 1617801 A	30-05-2001
WO 0136471	A	25-05-2001	AU 1769601 A	30-05-2001